

**MANGANESE RESPONSE AND  
NUTRIENT UPTAKE IN CONVENTIONAL AND GLYPHOSATE-RESISTANT  
SOYBEAN**

by

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## **Abstract**

Glyphosate-resistant (GR) soybean cultivars are widely accepted in the United States. Glyphosate-resistance provides many benefits to production agriculture, yet GR soybeans may require some additional management practices. The objectives of this research are to (a) determine response of GR and conventional (CV) soybean near isoline to manganese fertilization, (b) determine nutrient concentration and uptake in GR and CV soybean, (c) determine differences in yield of GR and CV soybean varieties, (d) quantifying Mn uptake when glyphosate is and is not applied to glyphosate-resistant soybean, and (e) determine glyphosate effect on soybean response to Mn treatments. A field study was conducted at 5 locations in Kansas from 2006 through 2007. Manganese soil test levels ranged from 4 to 52 mg Mn/kg. Soybean (near isoline) varieties were planted at each location in a split-block design with 4 replications. Manganese treatments consisted of soil-applied  $\text{MnSO}_4$  at 0, 2.8, 5.6, and 8.4 kg Mn/ha and foliar applied Mn at 0.22 and 0.45 kg Mn/ha. Leaf tissue and whole plant samples were taken at approximately R1, R3, and R6 growth stages and analyzed for N, P, K, Mn, and other nutrient concentrations. Few significant differences were found between varieties for concentration of any nutrient. Overall nutrient uptake under optimal growth conditions was greater in GR soybean than CV soybean varieties. There were no yield differences between GR and CV soybean varieties at low yielding locations ( $< 3.3$  Mg/ha). In high yielding environments, CV soybean yield was greater than GR soybean yield for the 0 kg Mn/ha rate. However, granular Mn additions increased yield of GR soybean but did not affect CV soybean yield while foliar Mn treatments at high yielding locations increased yields in GR and CV

soybean. In addition, a greenhouse study was conducted with a completely randomized block design having 5 blocks. Manganese treatments in the greenhouse study consisted of soil-applied  $\text{MnSO}_4$  at 0, 8.5, 17, and 25.5 mg Mn/pot and foliar applications of 0.66 and 1.33 mg Mn/pot. Treatments were with and without glyphosate applications. Glyphosate applications did not alter Mn concentrations or total Mn uptake in the soybean biomass.

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## **Dedication**

I dedicate this thesis to my parents who I strive to make proud. I miss and love you. Also, I dedicate my thesis to my son Martin who gave me the motivation to finish my writing.

# **CHAPTER 1 - A Literature Review of Genetic Modification for Glyphosate-resistant Soybean**

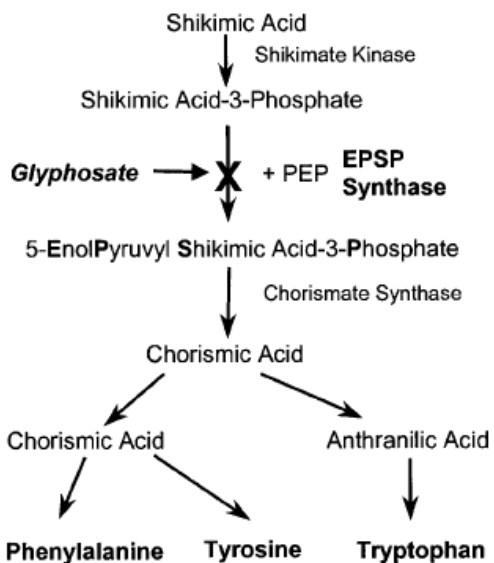
## **Introduction**

Soybean (*Glycine max L.*) is an important legume crop throughout the world. Soybean success as a major grain crop is due to the combination of agronomic benefits of soybean production and a diverse market for soybeans. Agronomic benefits of soybean production include greater drought tolerance in comparison to corn, additional weed control options, and N fixation from rhizobium symbiosis. These benefits have prompted significant research in developing new soybean varieties with improved genetics including soybean with specific tolerance to herbicide or higher genetic yield potentials (Gianessi and Carpenter, 2000).

One of the more notable advances in soybean genetics was the development of glyphosate-resistance. Use of glyphosate-resistant (GR) soybean has been widely adopted in the United States. Glyphosate is now the most commonly used herbicide in the United States (Gianessi and Reigner, 2005; Gianessi and Carpenter, 2000). It is a low cost, non-selective herbicide that was used in pre-cropping events until no-till cropping systems and introduction of glyphosate-resistance soybean and more recently corn, canola, and alfalfa (Cerdeira & Duke, 2006; Duke, 1988; Gianessi, 2000). In 2006, GR soybean occupied over 29 million ha in the United States alone (Monsanto, 2006). Glyphosate, first introduced in 1974 by Monsanto under the trade name Roundup, is a broad-spectrum amino acid synthesis inhibitor herbicide.

## *Glyphosate Mode of Action*

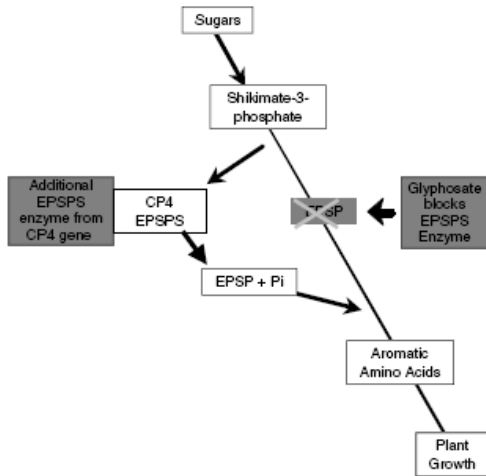
All soybean varieties must create enzymes, amino acids, and metabolites. Glyphosate interrupts the synthesis of enzyme 5-enolpyruvylshikimate-3-phosphate (EPSPS), or the shikimate pathway (Figure 1) (Dill, 2005). By inhibiting the EPSPS enzyme, aromatic amino acid production decreases and metabolic processes such as protein synthesis and photosynthesis are negatively affected (Dill, 2005; Duke et al., 2003). Glyphosate is the only herbicide that works by the inhibition of the shikimate pathway. Glyphosate causes a reduction in phytoalexins and increases some amino acids also released in root exudates. The infection of roots by soil borne organisms is a secondary mode of action for glyphosate (Kremer et al., 2005).



**Figure 1.1.** An illustration of the interruption of the shikimate pathway and where glyphosate interrupts its process. (Taken from Dill, 2005)

Glyphosate-resistant varieties are developed by the insertion of a bacterial-derived gene, CP4-EPSPS, into the DNA of conventional (CV) soybean varieties. Expression of this gene leads to glyphosate resistance by working around the glyphosate block of the EPSPS enzyme (Figure 2). Glyphosate will have minimal or no metabolization in the plant. Glyphosate moves quickly in

plant tissue and is translocated to metabolic sinks like nodules, seeds, and roots where the glyphosate can then be lost from the soybean system through root exudates (Dill, 2005; Kremer, 2005). However, Arregue et al. (2003) reported that glyphosate residual does persist in GR soybean leaves, stems, and grain.



**Figure 1.2.** Illustration of the technology for GR crops (Taken from Dill, 2005).

### *Glyphosate-resistant Technology Concerns*

With glyphosate introduction and expansion throughout the United States, several studies have been conducted comparing growth and management of GR and CV soybeans. Research by Elmore et al. (2001a and 2001b), Pline-Srnic (2005), Kremer (2005), Huber (2006), and Gordon (2005) have indicated possible reasons for additional management applications in GR soybean varieties. Glyphosate-resistant soybean may need to be managed differently than CV varieties based on a few theories, 1) when inserting the GR gene into the soybean, the genes controlling yield components may be disrupted, 2) the addition of the GR gene is extracting energy or carbon for its protein production rather than being used for seed production, 3) the GR gene may

be linked to a gene having a negative impact on soybean yield (Pline-Srnic, 2005), 4) GR gene introduction may alter the root exudates released from the GR soybean plant decreasing nutrient uptake (Kremer et al., 2005), and 5) glyphosate application may form insoluble nutrient complexes that are not available for plant uptake (Bernards et al., 2005).

Other concerns associated with GR soybean production include the potential negative effects from genetically altered crops due to the modifications of the plant genes or the glyphosate which exists in the seed that is used for animal feed, human food products, and supplements (Gianessi and Carpenter, 2000). In addition, the potential for creating GR weeds that would be difficult to control is a concern (Prowles and Preston, 2006). However, some of these drawbacks can be overcome by management practices, such as using crop rotations and alternate herbicides which will reduce the chances for glyphosate-resistance in weeds (Culpepper, 2006).

Genetic alteration in soybean has simplified some management strategies but posed questions concerning the potential need for additional management not necessary for CV soybean varieties. Few studies are reported comparing GR soybean production to CV varieties. Even fewer studies have reported results comparing near isogenetic soybean varieties for basic nutrient requirements, in which the genetic difference is glyphosate resistance. Extensive research is needed to determine if GR varieties may require different management than CV varieties. A review of the possible factors affecting growth and yield differences between GR and CV soybean is critical and management practices to overcome the potential genotypic differences need further investigation.

### ***Effect of Glyphosate on Glyphosate-resistant Soybean***

Elmore et al. (2001a) found that yields for their GR varieties were not affected by glyphosate rates up to twice the labeled use rate. Also, glyphosate applications during the vegetative and reproductive stages had no effect on yield and left the GR gene unchanged throughout following generations. Elmore et al. (2001a) reported that plant height and growth stages varied at 42 and 56 days after emergence when averaged over each location. Also differences in seed yield, plant height at 21 days after emergence, flowering date, plant height at flowering, date of physiological maturity, date of harvest maturity, lodging in 1999, growth stage at 42 days after emergence, and plant height at 56 days after emergence appeared between varieties from one location to the other in the 2-yr dataset (Elmore et al., 2001a). Glyphosate or ammonia sulfate (AMS) applications have not been found to alter soybean yield in any way.

Prostoko et al. (2003) found that at only one of their three locations glyphosate produced significant foliar damage to glyphosate-resistant soybean within 10 days after treatment (DAT). However, the chlorosis and leaf burn observed on the soybeans was not noticeable by 21 DAT. Others have found that glyphosate application to GR soybean decreased nodulation and nodule leghemoglobin content due to the buildup of shikimate in the soybean plant (Zablotowicz and Reddy, 2004).

### ***Glyphosate Secretion from Soybean Roots and Soybean Root Exudate Changes***

When glyphosate is applied to GR soybean it is released through the roots into the soil (Kremer et al., 2005). Furthermore, glyphosate used as a pre-emergence burn down herbicide can be exuded from target plants, stabilizing and altering interactions in the rhizosphere which can negatively

affect non-target plants (Neumann et al., 2006). In addition, glyphosate causes the infection of target plant roots by soil-borne microorganisms. This infection occurs because the target plant has decreased production of phytoalexins. Phytoalexins are plant protection compounds that are produced through the shikimate pathway. Once GR plants have glyphosate applied to them the glyphosate is either bound to the EPSPS pathway or translocated in the plant to accumulate in meristematic plant tissue (Eker et al., 2006).

Kremer et al. (2005) found that insufficient amounts of phytoalexins, which prevent fungal infections, are created in GR soybean. In addition, Kremer et al. (2005) found that GR soybean treated with or without glyphosate, had an increased amount of carbohydrates and amino acid in root exudates when compared to CV soybean varieties. Glyphosate appeared to increase some soil fungi in the rhizosphere possibly due to increased amino acid and carbohydrates in the root exudates. *Phytophthora* and *Fusarium* are two of the fungi most affected by glyphosate (Kremer et al., 2005). Increased fungi may have adverse affects on soybean plant growth, especially with GR soybean having inadequate production of phytoalexins. Nematode densities, soil microbial biomass, and substrate-induced respiration in soil were not affected by glyphosate when compared to other herbicide treatments (Liphadzi et al., 2005).

Glyphosate release through root exudates could also potentially have antagonistic effects with cations in either the soil solution or the plant. Glyphosate effectiveness can be altered by water quality, hardness, and cation content. Hard water cations, for example, iron, zinc, and calcium when mixed with glyphosate can create insoluble salt complexes that will not be easily absorbed into the plant (Nalewaja and Matysiak, 1991; Nalewaja and Matysiak, 1992). When this thought



is applied to the altering of root exudates it could indicate that glyphosate complexes manganese (Mn) in the soil rhizosphere making it less available for plant uptake.

Bailey et al. (2002) and Eker et al. (2006) reported that Mn added to herbicide solution caused reduced weed control due to the insoluble salt complexes formed which aren't readily absorbed by the plant. Bailey et al. (2002) found that when Mn was tank mixed with glyphosate and applied to GR soybeans it caused significant decreases in weed control, which could be overcome by using higher rates of herbicide for most weed species. Soybean yields were not affected by the glyphosate or the Mn (Bailey et al., 2002). Consequently, Mn could then become less available in the soil for plant uptake or made unavailable in the soybean plant for life cycle processes due to the antagonism between glyphosate and Mn (Kremer et al., 2005; Neumann et al., 2006).

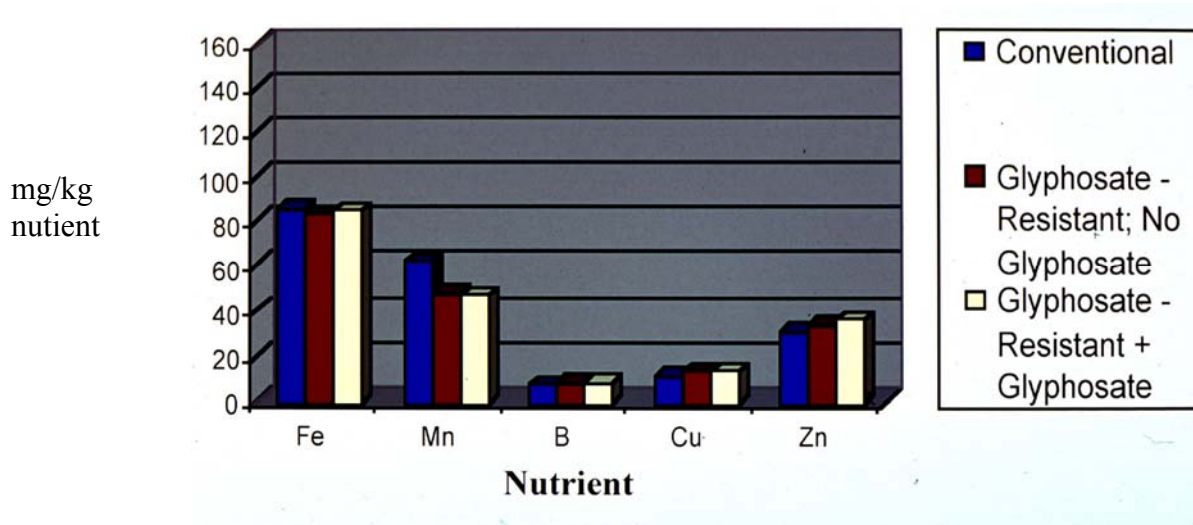
Eker et al. (2006) found that glyphosate application to sunflower, a non-target plant, had significant reductions of Fe and Mn concentrations in the leaf tissue of sunflower. In addition, the ratio of Mn-reducing to Mn-oxidizing bacteria has been found to be lower under GR soybean when no glyphosate is applied, and the ratio is even lower with glyphosate applications (Kremer, 2008).

### ***Huber's Research***

Don Huber (2006), at Purdue University, conducted preliminary research illustrated in Figure 1.3 showing that the CV varieties used in his research had greater concentrations of Mn in plant tissue than GR varieties. Manganese concentrations in tissue of the CV variety were about 20 mg/kg higher than the GR varieties when glyphosate was applied and when it wasn't applied.

Also, the glyphosate application did not seem to affect Mn concentrations in the leaf tissue (Figure 1.3). No significant differences were noticed for nutrients aside from Mn (Huber, 2006).

Huber (2006) found similar responses in GR corn.



**Figure 1.3.** Micronutrient concentrations in leaf tissue of CV soybeans, GR soybeans with glyphosate application, and GR soybeans without glyphosate application (Huber, 2006).

### Manganese Deficiency in Soybean

Some researchers support the idea that Mn needs are greater in GR soybean than they are in CV varieties. Manganese is critical for many physiological processes of the soybean plant. It is active in many cellular activities such as stabilization of structural proteins, the ultrastructure of chloroplasts, and photosynthesis. Manganese deficiency often causes decreases in soybean seed oil and increases in seed protein content (Wilson et al., 1982). Manganese can also be crucial for maximizing nitrogen fixation in soybeans under drought stressed conditions (Izaguirre-Mayoral, 2005). Manganese deficiency may cause a significant loss in yield.

Plant up take of Mn depends on the release of Mn from the solid phase into solution, Mn transport to the root surface by way of mass flow and diffusion, and the movement of Mn into the root following Michaelis-Menten kinetics (Sadana and Claassen, 2000). Altering enzymes in the root exudates could reduce Mn uptake in GR soybean varieties, thus creating a need for Mn supplements.

Visual Mn deficiency symptoms first occur as interveinal chlorosis, this is when the leaves are light green or yellow with dark veins. These symptoms will first appear on new foliage because Mn is somewhat immobile in plants; often plants will overcome this early deficiency on their own. If the deficiency is extreme, the new tissue will not recover and older tissue may also show deficiency symptoms (Heitholt et al., 2002). Critical leaf values for Mn in leaf tissue are between 16 to 22 mg Mn/kg dry plant matter (Ohki, 1974). Below the critical leaf value would be considered deficient. It is not until leaf tissue concentrations reach 200 mg Mn/kg plant tissue that Mn is considered toxic (Heitholt et al., 2002).

Many micronutrients, such as Mn, are limited on soils that are high in clay content, calcium, or soil pH (Heitholt et al., 2002). It is also common to have Mn deficiency on soils with high iron content (Mehlich, 1957). Critical values for soil Mn levels are between 12 and 15 mg/kg (Mascagni and Cox, 1985 and Ohki, 1974). However, researchers have had little success correlating soil extractable Mn levels to plant uptake (Reisenauer, 1998; Miyazawa et al, 1991).

A primary factor affecting Mn availability is soil pH. On soils with a pH greater than 7.0 and Mn soil concentrations below 10 mg/kg, soybeans will likely have a positive response to Mn

fertilizer applications (Cox, 1968). The higher the pH the less Mn is available. Mehlich (1957) reported that response to Mn supplements could be seen in soils with a pH of 6.2 and if Mn concentrations in the soil were less than 19 mg/kg. Often areas of the field such as tips of terraces, areas close to rock roads, and places with excessive lime deposits will have increased pH and Mn deficiency problems.

The levels of elements absorbed by the soybeans from the soil solution can vary greatly from different plant genotypes, cultural practices, and the soil and air environment. Moreover, plant tissue concentrations for elements are influenced by the interactions of many factors and do not directly reflect the plant needs. However, patterns of dry matter accumulation in specific parts of the plant are similar between varieties (Drossopoulos et al., 1994).

### ***Fertilizing Soybean for Manganese Deficiency***

Manganese deficiencies can be addressed in two general methods. Granular Mn fertilizer, such as Mn sulfate ( $\text{MnSO}_4$ ), can be broadcast, banded, or side dressed in the soybean field. Liquid Mn fertilizer, often as a manganese chelate, can be foliar-applied during the growing season. Foliar applications of liquid Mn fertilizer may need to be repeated multiple times to alleviate Mn deficiency (Cox, 1968). The timing of postemergence herbicides on soybeans coincides well with Mn applications and glyphosate could be acceptable to tank mix with Mn applications (Heckman, 1999).

Boswell et al. (1981) reported a visual response to Mn application on deficient plants at the V4 growth stage. They also found that they could apply too much Mn and induce Mn toxicity in the

plants (Boswell et al., 1981). Manganese toxicity can be visually identified as crinkling of the leaf, interveinal chlorosis, necrotic spotting of the leaves, and malformation of the pods. If the deficiency is induced late in the growing stages, often because of wet growing conditions, the pods and yield may not be greatly affected (Parker et al., 1969).

Boswell et al. (1981) did not find any significant influence of application method of Mn between side dressing, banding, broadcast, or foliar treatments. However, trends suggested that side dressing Mn sulfate at planting had the lowest yields. As more Mn was applied, Mn concentrations in the plant leaves and seeds increased. However, yields did not directly increase with higher Mn rates. Soybean yield was best correlated with Mn concentrations in soybean seed rather than leaf tissue. The best correlation between Mn concentrations in leaf tissue and yield were found from samples collected at the R6 growth stage. Their data suggested a critical seed concentration value of 16 mg/kg Mn (Boswell et al., 1981).

In a greenhouse study, Heitholt et al. (2002) found that Mn fertilization increased chlorophyll content during later stages of development. They also found at growth stage R6, leaf blade Mn concentrations in Mn treated soybeans were higher than the 0 mg Mn/ha control. The additions of Mn increased the number of seeds per plant from 53, at 0 ppm Mn, to 77 ( $P=0.02$ ), when averaged across the 10 mg Mn/kg, 20 mg Mn/kg, 30 mg Mn/kg, and 40 mg Mn/kg rates. Manganese concentrations in tissue of plants reached 132 mg Mn/kg which is above the critical value of 17 mg Mn/kg despite soil conditions typical of Mn deficiencies (Heitholt et al., 2002).

Mascagni and Cox (1985) found broadcasting Mn increased soil test Mn, leaf tissue Mn, and soybean yields in Mn deficient conditions. It was also reported that when using foliar Mn fertilizer, the maximum yield was achieved when applications were made at the first sign of deficiency, which were at the V4 growth stage in this study. There was little benefit from foliar applications made late in the season. They also found that making two spray applications at V4 and R1 of Mn was just as good as three spray applications throughout the growing season (Mascagni and Cox, 1985).

### **Comparing Glyphosate-resistant and Conventional Soybean**

Although some research suggests that glyphosate application is responsible for the inhibition of Mn uptake in GR soybean, other studies suggest that Mn uptake in GR soybean is less than CV soybean because of genetic modifications required for the glyphosate resistance. Comparison of GR soybean and CV soybean varieties can be a difficult process. Early in the introduction of glyphosate-resistance technology, the primary debates were about appropriate herbicide use, therefore, variety testing was not set up to compare GR and CV soybean varieties.

In initial variety trials for GR soybean, CV soybean varieties were used as checks for GR soybean plots. Raymer (1997) reported that some GR soybean varieties did yield greater than the overall study mean with CV and GR soybean, but the mean yield of GR varieties used in his trial was significantly lower than the overall study mean. Also, mean CV soybean yield in the variety trial was significantly greater than the overall study mean. Furthermore, research indicated that when the top 5 GR soybean variety yields were compared to the top 5 CV soybean variety yields, the CV soybean yields were greater (Oplinger et al., 1998). However, Delanney et al. (1995) did

not find any significant differences between yields of CV and GR soybean varieties in data from over 60 field trials.

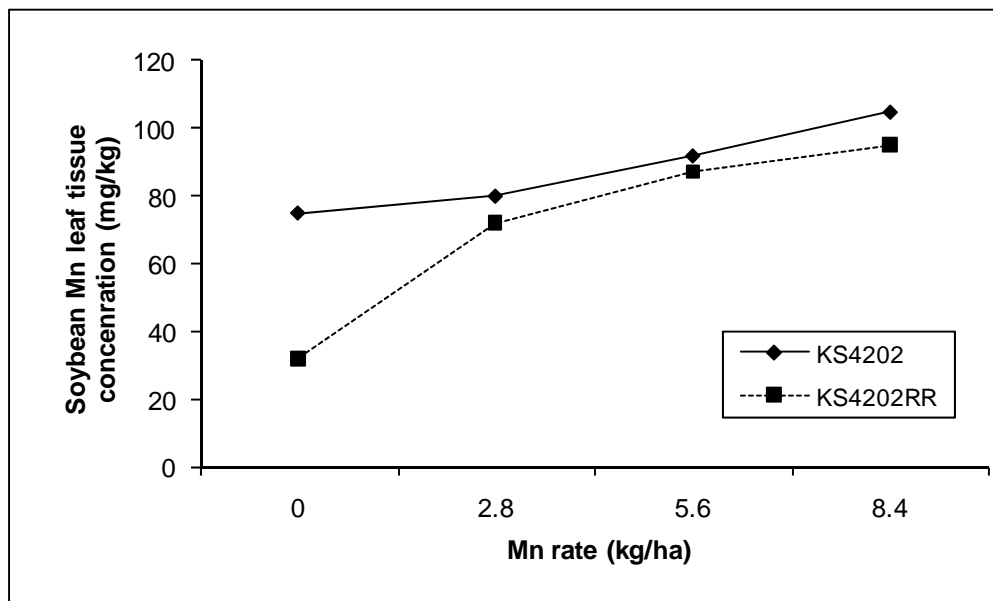
### ***Crop Growth and Yield of Soybean Near Isolines***

Despite field trial evidence that GR soybean yields were equal to CV soybean yields (Delanney et al., 1995), many researchers felt that evidence of a possible yield drag from GR technology does or did exist. A yield drag has been observed with GR soybean varieties when glyphosate is not applied (Huber, 2006; Gordon, 2005; Elmore et al., 2001a). Elmore et al. (2001b) compared GR varieties and high yielding CV varieties and found that the GR varieties yielded 10% less than the high yielding CV varieties. It was also determined that GR varieties, on average, yielded 5% less than their CV sister line or near isogenetic variety (Elmore et al., 2001a). In addition, differences were also noted in seed weight and plant height between sister lines. Elmore et al. (2001b) attributed the yield and growth differences to the insertion of the CP4-ESPS gene or the insertion process.

### ***Gordon's Research***

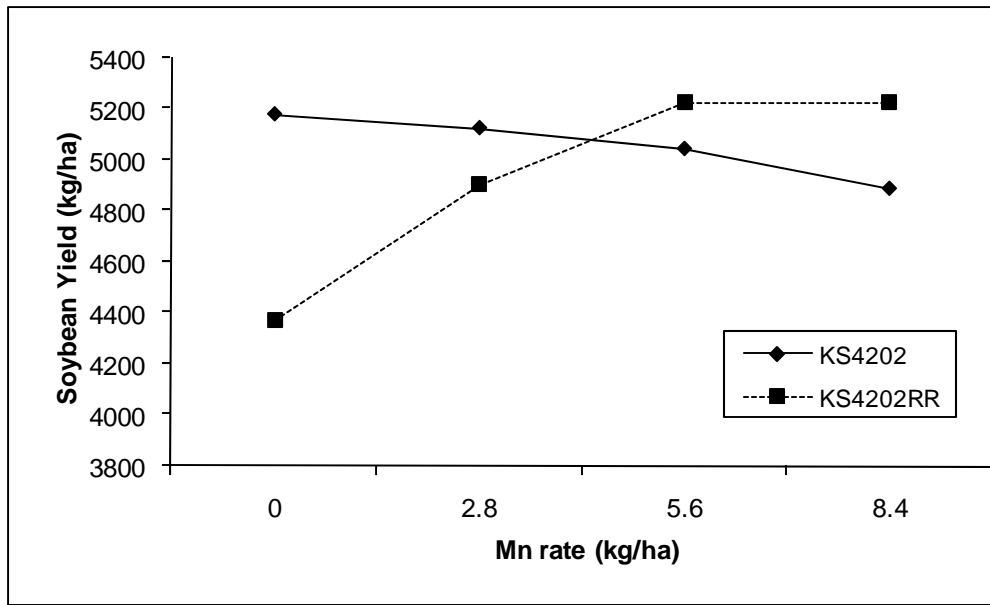
In 2005, a study was initiated at the North Central Kansas Experiment Field to determine if GR soybeans will respond differently than CV soybeans to soil applied and foliar Mn treatments (Gordon, 2005). Two near isoline, cultivar KS4202 and variety K4202RR, were planted with a range of Mn rates applied as sub-surface banded Mn sulfate or foliar-applied EDTA-Mn (Mn chelate MnEDTA, Claw EL). The Mn sulfate was applied in a subsurface band at planting. The foliar applications were made with a backpack sprayer at the V6 stage. Leaf tissue samples were taken from all treatments at the full bloom stage. No glyphosate was applied to either of the soybean varieties.

Manganese sulfate additions increased both tissue concentration (Figure 1.4) and yield (Figure 1.5) of the GR soybeans (Gordon, 2005). Manganese additions also increased tissue concentrations in the CV soybean variety, yet no yield increase was observed. Tissue concentration at full bloom in the GR variety was less than half of what was found present in the CV variety when no Mn was added. Results from the first year of data indicated that the CV variety yielded 809 kg/ha greater than its GR isolate when no Mn was added. With Mn additions, the tissue concentrations and yield for the GR soybeans equaled that of the CV soybeans (Gordon, 2005).



**Figure 1.4.** Manganese concentrations in conventional soybean KS4202 and glyphosate-resistant soybean K4202RR leaf tissue in mg/kg at four rates of soil-applied Mn sulfate (Gordon, 2005).





**Figure 1.5.** Soybean yield for conventional KS 4202 and glyphosate-resistant K4202RR at four rates of soil-applied Mn sulfate (Gordon, 2005).

## Conclusions

Yield drag and low Mn concentrations in plant tissue have been observed with GR varieties (Elmore et al., 2001b; Huber, 2006; Gordon, 2005). Elmore et al. (2001a) found that GR varieties, on average, yielded 5% less than their CV sister line. In addition, differences were also noted in seed weight and plant height between sister lines. Elmore et al. (2001b) also found that GR varieties were not affected by glyphosate when applied at rates up to twice the labeled use rate. Glyphosate applications during the vegetative and reproductive stages had no effect on yield or Mn concentration, and left the GR gene unchanged throughout following generations.

Very little research has been completed comparing GR and CV soybeans. Even less has been reported on why GR soybeans may have lower yields and what management practices could be used to overcome the yield lag. It is important that all possibilities are explored. Since very little information is available for Mn fertilization in Kansas research is needed to determine if Kansas

agricultural producers could benefit from Mn fertilization and what methods would be best to alleviate Mn deficiencies. The objectives of this research are to (a) determine response of GR and CV soybean varieties to foliar- and soil-applied manganese fertilization, (b) determine nutrient concentration and uptake in GR and CV soybean at different growth stages, (c) determine differences in yield of soybean near isoline varieties (with and without glyphosate-resistance), (d) quantifying Mn uptake when glyphosate is and is not applied to glyphosate-resistant soybean, and (e) determine glyphosate effect on soybean response to foliar- and soil-applied Mn treatments.

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## **CHAPTER 2 - Glyphosate-resistant and Conventional Soybean**

### **Response to Manganese Fertilizer**

#### **Introduction**

Research by Elmore et al. (2001a and 2001b), Pline-Srnic (2005), and Kremer et al. (2005) have indicated that additional management practices may be necessary for glyphosate-resistant (GR) soybean varieties. Glyphosate-resistant soybean varieties may need to be managed differently than conventional (CV) varieties based on a few theories, 1) when inserting the GR gene into the soybean, the genes controlling yield components may be disrupted, 2) the GR gene is stealing energy or carbon for its protein production rather than being used for seed production, 3) the GR gene may be linked to a gene having a negative impact on soybean yield (Pline-Srnic, 2005), 4) GR gene introduction may alter the root exudates released from GR soybean plants, thus decreasing nutrient uptake (Kremer et al., 2005), and 5) glyphosate may form insoluble complexes with some nutrients, thus interfering with nutrient uptake or metabolism (Bernards et al., 2005).

Limited research is available analyzing GR soybean root exudates and rhizosphere microbial activity when glyphosate is not applied to GR soybean varieties but Gressel (2002) suggests that the GR gene is less efficient than the standard enzyme 5-enylpyruvylshikimate-3-phosphate (EPSPS) gene. The EPSPS gene is critical aromatic amino acids production and metabolic processes such as protein synthesis and photosynthesis (Dill, 2005). Gressel (2002) also found

that GR soybean produce insufficient amounts of phytoalexins, which are antibiotics that prevent fungal infections. Kremer et al. (2005) found that GR soybean treated with or without glyphosate, had an higher carbohydrate and amino acid contents in root exudates than CV soybean. Furthermore, the ratio of Mn-reducing to Mn-oxidizing bacteria has been found to be lower for GR soybean compared to conventional soybeans (Kremer, 2008)

Several studies have documented glyphosate release through roots of plants that have been sprayed with glyphosate (Kremer et al., 2005; Neumann et al., 2006). Glyphosate release from roots of treated plants can alter microbial activity and composition of the rhizosphere. For example, Zablotowicz and Reddy (2004) found that glyphosate application to soybean inhibits the *Bradyrhizobium japonicum* symbiosis. Kremer et al. (2005) found that glyphosate exuded through the roots stimulated rhizosphere fungi, possibly having adverse affects on plant growth and biological processes. Kremer and Means (2006) found that Fusarium root colonization was increased when glyphosate applications are made to GR soybean. Even though the ratio of Mn-reducing to Mn-oxidizing bacteria is lower for GR soybean in absence of glyphosate application, the ratio is further reduced with glyphosate applications (Kremer, 2008). Glyphosate used as a pre-emergence burn down herbicide would also be exuded from target plants, stabilizing, and altering interactions in the rhizosphere which can negatively affect non-target plants (Neumann et al., 2006).

Bernards et al. (2005) found that glyphosate efficacy was at times reduced due to foliar Mn fertilizer additions. When glyphosate is exuded though the roots into the soil, it is then possible that Mn in the soil could become less available for plant uptake due to the formation of insoluble

Mn-glyphosate complexes (Kremer et al., 2005; Neumann et al, 2006). Baliey et al. (2002) and Eker et al. (2006) reported that manganese added to herbicide solution reduced weed control due to the insoluble salt complexes formed, which were not readily absorbed by the plant.

Altering enzymes or root exudates could reduce Mn uptake in GR soybean varieties by creating cation complexes in the soil solution or in the plant itself. This may create a need for Mn supplements. However, researchers have had little success correlating soil extractable Mn levels to plant uptake (Reisenauer, 1998; Miyazawa et al, 1991). Plant up take of Mn depends on the release of Mn from the solid phase into solution, Mn transport to the root surface by way of mass flow and diffusion, and the movement of Mn into the root following Michaelis-Menten kinetics (Sadana and Claassen, 2000).

The DTPA soil test (Lindsay and Norvell, 1978) is used to determine Zn, Fe, Mn, and Cu concentrations in near-neutral or calcareous soils. Research indicates that soil moisture, temperature, sun light, organic matter content, and sample handling all can have an impact on Mn soil test results (Andrade et al., 2005). Lindsay and Norvell (1978) also report that pH, concentration of chelating agent, shaking time, and the temperature of the extraction can affect the sensitive micronutrient concentration.

Manganese deficiency may cause a significant yield loss. Manganese deficiency will also decrease soybean seed oil and increase seed protein content (Wilson et al., 1982). Critical leaf values for manganese in leaf tissue are between 16 to 22 mg Mn/kg dry plant matter (Ohki et al, 1979; Mills and Jones, 1991), concentrations below the critical leaf value would be considered

deficient. It is not until leaf tissue concentrations reach 200 mg Mn/kg plant tissue that manganese is considered toxic (Heitholt et al., 2002).

Manganese deficiencies can be addressed in two general methods. Granular Mn fertilizer, such as Mn sulfate ( $\text{MnSO}_4$ ), can be broadcast, banded, or side dressed in the soybean field. Liquid manganese fertilizer, often as a manganese chelate, can be foliar-applied during the growing season. Foliar-applied liquid manganese fertilizer may need multiple applications to alleviate manganese deficiency (Cox, 1968). Often post-emergence glyphosate applications coincide with foliar Mn fertilizer applications.

With the alteration of genetics in GR soybean causing changes in root exudates and possible interactions with glyphosate in the rhizosphere, it is critical to determine if Mn uptake of GR soybeans have been negatively affected. Gordon (2005) and Huber (2006) have both found that some GR soybean have reduced Mn concentrations in leaf tissue. In addition, Gordon (2005) found that some GR soybean have reduced yields compared to CV near isoline. The objective of this research is to (a) determine response of GR and CV soybean varieties to foliar- and soil-applied manganese fertilization.

## Materials and Methods

In 2005 a field study was initiated in Scandia, KS. In 2006 and 2007 the study was extended to Ashland Bottoms, Rossville, Manhattan, and Ottawa, KS (not all locations were used in both years). Soil samples were taken prior to planting and analyzed for P, K, pH, organic matter (OM), Mn, and Fe (Table 2.1). Soil pH was determined by the SMP buffer method (Shoemaker et al., 1961), soil test P concentration was determined by the Mehlich III extraction (Mehlich, 1984), exchangeable K was extracted with ammonium acetate (Thomas, 1982), Fe and Mn were extracted with the DTPA extraction (Lindsay and Norvell, 1978), and organic matter percent was determined by the Walkley-Black procedure (Walkley, 1947).

**Table 2.1.** Select soil analysis from field locations for 2006 and 2007.

Year	Location	pH	om <sup>†</sup>	Mn	P	K	Fe
2006					----- mg/kg -----		
	Ashland Bottoms	6.5	2.2	23	33	388	44
	Rossville	7.1	1.8	14	16	155	20
	Scandia	6.7	2.7	15	64	438	25
	Ottawa	5.9	2.5	51	30	154	56
2007	Ashland Bottoms	6.3	2.9	13	16	194	82
	Rossville	6.3	2.3	31	27	319	42
	Scandia	6.0	2.7	26	8	438	64
	Manhattan	8.3	0.9	4	68	132	9

<sup>†</sup> organic matter

Soil samples were all handled similarly and dried at 60° C within 4 hrs of sampling to reduce procedural variability, which may alter Mn test results (Andrade et al., 2005; Lindsey and Norvell, 1978). Predominant soil series at each location are as follows, Rossville: Muscotah silty clay loam (fine, smectic, mesic Aquertic Hapludolls); Ashland Bottoms: Bismarckgrove-Kimo Complex (fine-silty, mixed, superactive, mesic, Fluventic Hapludolls – clayey over loam,

smectic, mesic, Fluvenaquentic Hapludolls); Ottawa: Woodson silt loam (fine, smectic, thermic Abruptic Argiaquolls); Manhattans: Zeandale silt loam (coarse-silty, mixed, superactive, calcareous, mesic Typic Udifluvents); and Scandia: Crete silt loam (fine, smectic, mesic Pachic Arguistolls).

The experimental design was a randomized, split block design with near isoline as the whole plot factor and Mn treatment as the sub plot factor. There were 4 replications at each location except Scandia in 2006, where there were only 3 replications. Plots were 3.1 m wide by 10.7 m long, row spacing was 0.8 m. Soybean near isolines, cultivar KS4202 and variety K4202RR or cultivar KS4602N and variety K4602NRR (when soybean cyst nematode was a concern or there was a difficulty of obtaining an isoline) were planted at a seeding rate of approximately 290,000 seeds/ha (Table 2.2). Manganese treatments included a soil-applied surface band of MnSO<sub>4</sub> at 0, 2.8, 5.6, and 8.4 kg Mn/ha (Mn was applied in a subsurface band at Scandia in 2006 and 2007) and foliar applications of 0.22 and 0.45 kg Mn/ha. Manganese chelate (Mn-EDTA) was applied with a backpack sprayer at growth Stage R1 after the first tissue samples taken.

**Table 2.2.** Soybean near isoline and planting dates for each study location in 2006 and 2007.

	Location	Near Isolines	Planting Date (mm.dd.yyyy)
2006	Ashland Bottoms	KS4202 & K4202RR	05.17.2006
	Rossville	KS4602N & K4602NRR	05.16.2006
	Scandia	KS4202 & K4202RR	05.15.2006
	Ottawa	KS4202 & K4202RR	05.22.2006
2007	Ashland Bottoms	KS4202 & K4202RR	05.18.2007
	Rossville	KS4602N & K4602NRR	05.22.2007
	Scandia	KS4202 & K4202RR	05.21.2007
	Manhattan	KS4602N & K4602NRR	06.04.2007

Glyphosate was not applied during the growing season but it was used as a pre-plant burn down herbicide at most locations (Table 2.3). Pre-emerge herbicides and manual weeding were used to control weeds during the crop growing season. All locations were irrigated except Ottawa to maintain adequate soil moisture. Additional fertilizers were not used except at Rossville and Scandia where a starter fertilizer was used (Table 2.3).

**Table 2.3.** Cultural practices for tillage, herbicide, irrigation, and fertilizer for Mn study.

Location	Year	Tillage	Herbicide	Irrigation	Fertilizer
Ashland Bottoms	2006	Conventional	None	Furrow	None
Ashland Bottoms	2007	Conventional	Pre-plant: Round-up, Dual, and Authority First	Furrow	None
Rossville	2006	Conventional	Flexstar and Select	Sprinkler	Starter
Rossville	2007	Conventional	Pre-plant: Round-up, Dual, and Authority First	Sprinkler	Starter
Scandia	2006	No-till	Pre-plant: Round-up,	Sprinkler	Starter
Scandia	2007	No-till	Pre-plant: Round-up,	Sprinkler	Starter
Ottawa	2006	No-till	Flexstar and Select	None	None
Manhattan	2007	Conventional	Pre-plant: Round-up, Dual, and Authority First	Sprinkler	None

In general, each location had normal to low average monthly precipitation during the growing season compared to 30 year normals. The Ashland Bottoms and Manhattan locations received large rainfall events in August 2006 and May 2007 which brought monthly averages well above normal. Overall, monthly average maximum temperatures were higher than 30 year normals at all locations except Rossville, Ashland Bottoms, and Manhattan which were normal in 2007. Growing-season monthly average minimum temperatures were cooler than normal at all locations in each year compared to 30 year normal minimum conditions (Appendix A).

Representative leaf tissue samples were taken from every plot at approximate growth stages R1, R3, and R6 from the upper most fully developed trifoliolate. Plant height was recorded at all three growth stages. Thirty feet of the center two rows in each plot was harvested at maturity for grain yield using a plot combine. A plot combine was not available at the 2007 Manhattan location, therefore, 20 ft of the center two rows were hand harvested and threshed with an Almaco thresher.

Tissue and grain samples were oven dried at 60° C then prepared for digestion by grinding to pass a 2 mm sieve with a Wiley mill. Tissue samples were digested with a nitric acid according to the following procedure. A 0.50-g sub-sample of each tissue sample was weighed into digestion tubes. Ten ml of concentrated nitric acid was added to each digest tube and allowed to sit over night. Tubes were then placed on a digestion block at 127° C for a 1 hour digest. Twenty ml of 30% hydrogen peroxide was added to each sample and returned to the digestion block for 2 hours at 127° C. Each digestion tube then received 50 ml of 20% HCl and was brought to volume



(100 ml) with deionized water. Manganese concentration of digests was determined with an ICP spectrometer.

Treatment effects were determined by ANOVA and planned treatment comparisons were done with the protected LSD multiple comparisons procedure. Statistical computations were completed with SAS proc mixed models procedures. Each year and location was analyzed separately due to location variations. Repetition was considered a random variable with near isoline and rate being fixed effects (Appendix B).

## **Results and Discussion**

### ***Soybean Tissue Manganese Concentrations***

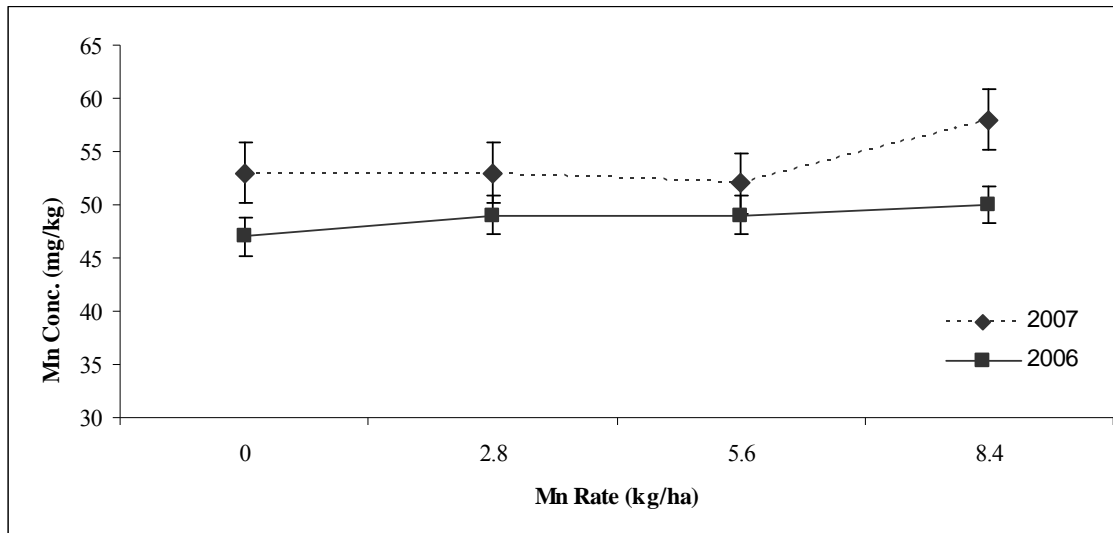
#### ***Growth Stage R1***

At growth stage R1, Mn concentration in plant tissue was significantly affected by soybean near isoline in 2006 at Rossville, with GR soybean having 8 mg Mn/kg more than CV soybean when averaged over Mn treatments (Table 2.4). Contrary to Rossville in 2006, CV soybean at Ashland in 2007 resulted in 5 kg Mn/ha more than GR soybean when averaged over Mn rates. In addition, increasing Mn rate to 8.4 kg Mn/ha with granular fertilizer increased Mn tissue concentration at Ashland in 2007 (Figure 2.1).

**Table 2.4.** Analysis of variance (ANOVA) p-values from F-tests for near isolate (GR and CV) and Mn rate (foliar and granular Mn fertilizer) effects on Mn concentration at growth stage R1.

		Ashland Bottoms	Rossville	Scandia	Ottawa	Manhattan
2006	near isolate	0.392	0.002	0.190	0.816	NA <sup>†</sup>
	rate	0.528	0.193	0.180	0.905	NA <sup>†</sup>
	near isolate*rate	0.701	0.486	0.318	0.044	NA <sup>†</sup>
2007	near isolate	0.003	0.792	0.735	NA <sup>†</sup>	0.445
	rate	0.002	0.333	<0.0001	NA <sup>†</sup>	0.742
	near isolate*rate	0.676	0.900	0.559	NA <sup>†</sup>	0.302

† Data not available



**Figure 2.1.** Manganese concentrations in soybean leaf tissue at growth stage R1 as affected by different rates of soil-applied MnSO<sub>4</sub> at Ashland Bottoms for the 2006 and 2007 growing seasons (Error bars are standard errors generated by SAS, data averaged across near isolines).

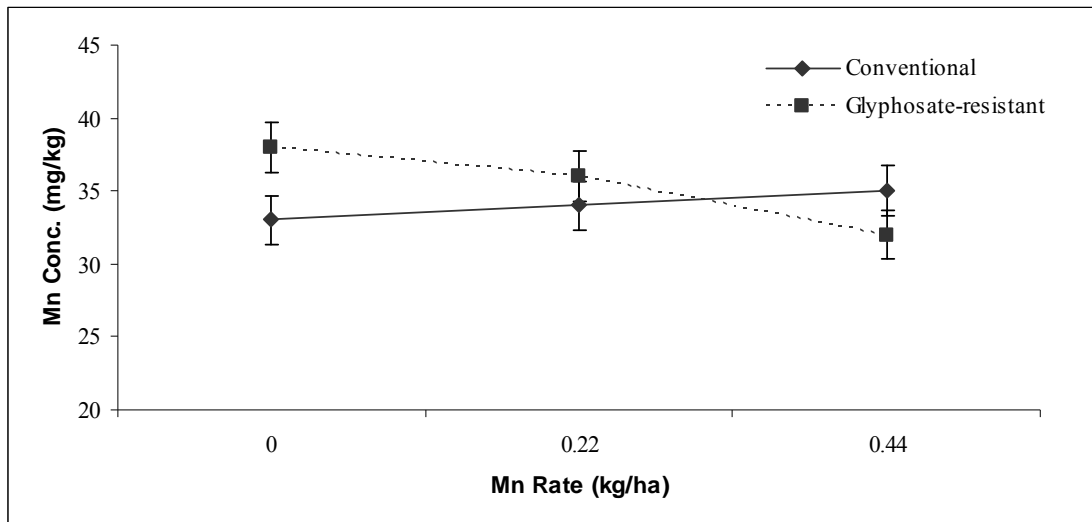
A significant near isolate by Mn rate interaction at Ottawa occurred in 2006 with GR soybean leaf tissue Mn concentrations being higher than CV soybean leaf tissue Mn concentration in the control plots (Table 2.5). However, Mn treatments did not increase tissue Mn concentrations of either near isolate (Figures 2.2 & 2.3). At Scandia in 2007 a significant Mn rate effect was

found, although the rate effect was a result of unusually high Mn concentrations in some foliar treatments (Table 2.5). In contrast to all other locations, tissue samples from this site were collected after foliar Mn treatments were applied, which is the most likely cause of the observed interaction.

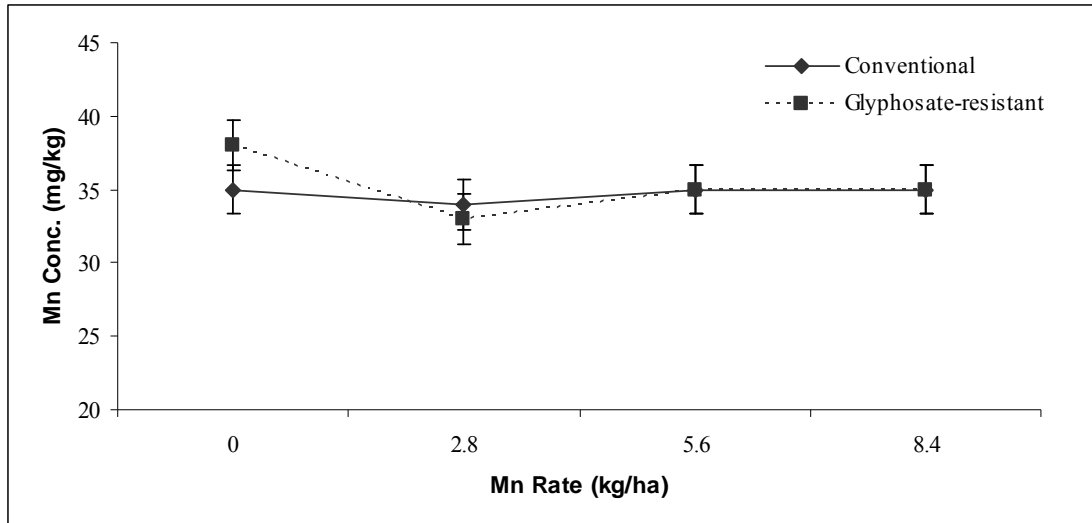
**Table 2.5.** Manganese tissue concentrations in conventional (CV) and glyphosate-resistant (GR) soybean at growth stage R1 (LSD for Mn rate by soybean near isoline interactions).

Year	Mn Rate	mg/kg											
		Ashland		Rossville		Scandia		Ottawa		Manhattan			
		Bottoms		CV	GR	CV	GR	CV	GR	CV	GR	CV	GR
2006	0	45.5	49.0	48.5	60.7	43.1	54.5	33.0	37.5	NA <sup>†</sup>	NA <sup>†</sup>		
	0.22	47.4	49.5	49.2	55.2	NA <sup>†</sup>	NA <sup>†</sup>	33.7	36.0	NA <sup>†</sup>	NA <sup>†</sup>		
	0.44	48.5	50.7	48.0	52.2	NA <sup>†</sup>	NA <sup>†</sup>	34.7	32.7	NA <sup>†</sup>	NA <sup>†</sup>		
	2.8	48.5	49.7	52.0	69.5	47.1	57.0	36.2	32.5	NA <sup>†</sup>	NA <sup>†</sup>		
	5.6	46.5	50.5	50.2	53.2	45.9	51.5	34.7	34.5	NA <sup>†</sup>	NA <sup>†</sup>		
	8.4	48.2	51.2	50.5	55.2	52.6	55.0	34.2	34.7	NA <sup>†</sup>	NA <sup>†</sup>		
LSD (0.05)		NS		NS		NS		1.7					
2007	0	54.7	50.5	67.5	63.2	67.0	75.5	NA <sup>†</sup>	NA <sup>†</sup>	122.7	108.5		
	0.22	55.0	50.5	62.0	61.2	390.0	367.5	NA <sup>†</sup>	NA <sup>†</sup>	111.2	108.1		
	0.44	52.7	50.0	59.7	65.0	475.0	375.0	NA <sup>†</sup>	NA <sup>†</sup>	100.5	122.4		
	2.8	54.0	52.2	63.5	65.0	67.5	71.0	NA <sup>†</sup>	NA <sup>†</sup>	130.9	106.7		
	5.6	54.5	50.5	63.5	67.2	92.5	67.0	NA <sup>†</sup>	NA <sup>†</sup>	125.2	119.0		
	8.4	58.2	58.2	70.2	71.5	68.3	96.5	NA <sup>†</sup>	NA <sup>†</sup>	124.8	79.69		
LSD (0.05)		NS		NS		NS				NS			

† Data not available



**Figure 2.2.** Effects of foliar Mn treatments on Mn concentrations in leaf tissue of conventional and glyphosate-resistant soybean near isoline at growth stage R1 for the 2006 Ottawa location. (Error bars are standard error generated by SAS)



**Figure 2.3.** Effects of soil-applied  $\text{MnSO}_4$  treatments on Mn concentrations in leaf tissue of conventional and glyphosate-resistant soybean near isoline at growth stage R1 for the 2006 Ottawa location. (Error bars are standard error generated by SAS)

### *Growth Stage R3*

The only significant treatment effects on Mn concentration in leaf tissue at growth stage R3 were rate effects at Ashland Bottoms and Manhattan (Table 2.6). Soybean leaf tissue at both locations had significantly higher Mn concentrations when Mn was foliar-applied (Table 2.7). However, soil-applied Mn did not seem to increase Mn concentrations in leaf tissue. The near isoline\*rate interaction was close to significant for the 2006 Scandia location ( $p=0.057$ ), where soil-applied  $\text{MnSO}_4$  tended to increase Mn concentrations in tissue of CV soybean but did not affect Mn concentrations in tissue of GR soybean (Table 2.7). Similar to the majority of our results, Heitholt et al. (2002) reported that soil-applied  $\text{MnSO}_4$  rate did not affect Mn leaf concentration at growth stage R3.

**Table 2.6.** Analysis of variance (ANOVA) p-values from F-tests for near isoline (GR and CV) and Mn rate (foliar and granular Mn fertilizer) effects on Mn concentration at growth stage R3.

		Ashland Bottoms	Rossville	Scandia	Ottawa	Manhattan
2006						
	near isoline	0.421	0.546	0.969	0.705	NA <sup>†</sup>
	rate	0.234	0.710	0.154	0.770	NA <sup>†</sup>
	near isoline*rate	0.512	0.476	0.057	0.478	NA <sup>†</sup>
2007						
	near isoline	0.748	0.531	0.785	NA <sup>†</sup>	0.783
	rate	0.024	0.978	0.778	NA <sup>†</sup>	<0.001
	near isoline*rate	0.955	0.640	0.903	NA <sup>†</sup>	0.616

† Data not available

**Table 2.7.** Manganese concentrations in soybean leaf tissue at growth stage R3 for conventional (CV) and glyphosate-resistant (GR) near isoline. (LSD for Mn rate by soybean near isoline interactions).

Year	Mn Rate	Ashland Bottoms		Rossville		Scandia		Ottawa		Manhattan	
		CV	GR	CV	GR	CV	GR	CV	GR	CV	GR
----- mg/kg -----											
2006											
	0	75.0	72.5	62.5	59.5	64.8	71.0	49.0	48.0	NA <sup>†</sup>	NA <sup>†</sup>
	0.22	71.0	67.7	62.0	65.7	NA <sup>†</sup>	NA <sup>†</sup>	46.0	49.0	NA <sup>†</sup>	NA <sup>†</sup>
	0.44	64.0	69.0	63.5	60.0	NA <sup>†</sup>	NA <sup>†</sup>	46.0	46.5	NA <sup>†</sup>	NA <sup>†</sup>
	2.8	76.0	69.0	59.7	61.5	69.6	74.0	48.2	44.0	NA <sup>†</sup>	NA <sup>†</sup>
	5.6	71.7	73.0	62.5	59.0	71.0	69.0	46.2	46.2	NA <sup>†</sup>	NA <sup>†</sup>
	8.4	71.7	73.7	61.2	61.0	73.7	71.0	48.0	45.2	NA <sup>†</sup>	NA <sup>†</sup>
LSD (0.05)		NS		NS		NS		NS			
2007											
	0	63.0	62.0	59.0	59.0	101.5	93.0	NA <sup>†</sup>	NA <sup>†</sup>	86.9	89.0
	0.22	66.0	66.7	58.5	60.5	88.4	89.1	NA <sup>†</sup>	NA <sup>†</sup>	131.3	172.7
	0.44	56.2	56.2	58.0	58.5	81.5	73.2	NA <sup>†</sup>	NA <sup>†</sup>	249.5	228.0
	2.8	63.7	63.5	59.5	58.0	89.0	94.3	NA <sup>†</sup>	NA <sup>†</sup>	104.0	88.1
	5.6	63.0	67.2	56.7	63.7	89.9	99.3	NA <sup>†</sup>	NA <sup>†</sup>	116.5	92.0
	8.4	62.7	63.0	58.5	60.2	95.4	88.8	NA <sup>†</sup>	NA <sup>†</sup>	85.5	73.3
LSD (0.05)		NS		NS		NS				NS	

† Data not available

**Growth Stage R6**

Manganese concentrations in plant tissue were not significantly affected by treatments at any location in either year (Appendix C). Once plants had reached the latter reproductive stages and were mostly mature the Mn concentrations had leveled out so that concentrations differences were not evident. Unlike previous research (Boswell et al., 1981; Heitholt et al., 2002) where Mn tissue concentrations increased with Mn fertilizer additions, we did not find Mn application effects on Mn concentration in soybean leaf tissue at growth stage R6.

### ***Plant Growth and Manganese Concentrations in Soybean Seed***

Glyphosate-resistant soybean was taller than CV soybean varieties at growth stage R6 at Ashland Bottoms in 2006, which is similar to results of Elmore et al. (2001a). However, at Scandia during 2007 CV soybean was taller (Appendix D). Manganese concentrations in harvested soybean seed were significantly affected by near isoline at Ashland Bottoms in 2006 (Table 2.8), where CV soybean contained 2 mg Mn/kg more Mn than GR soybean (Table 2.9). Seed Mn concentrations were not significant affected by Mn rate neither were there any Mn rate by near isoline interactions at any location in either year (Table 2.9).

**Table 2.8.** Analysis of variance (ANOVA) p-values from F-tests for near isoline (GR and CV) and Mn rate (foliar and granular Mn fertilizer) effects on seed Mn concentration at harvest.

	Ashland Bottoms	Rossville	Scandia	Ottawa	Manhattan
2006					
near isoline	<0.001	0.513	NA <sup>†</sup>	0.261	NA <sup>†</sup>
rate	0.776	0.908	NA <sup>†</sup>	0.576	NA <sup>†</sup>
near isoline*rate	0.966	0.683	NA <sup>†</sup>	0.576	NA <sup>†</sup>
2007					
near isoline	0.087	0.315	NA <sup>†</sup>	NA <sup>†</sup>	0.261
rate	0.637	0.868	NA <sup>†</sup>	NA <sup>†</sup>	0.576
near isoline*rate	0.742	0.678	NA <sup>†</sup>	NA <sup>†</sup>	0.575

† Data not available

**Table 2.9.** Manganese seed concentrations at harvest (LSD for Mn rate by soybean near isoline interactions).

Year	Mn Rate	mg/kg									
		Ashland Bottoms		Rossville		Scandia		Ottawa		Manhattan	
		CV	GR	CV	GR	CV	GR	CV	GR	CV	GR
2006											
	0	42.25	39.00	26.00	26.50	NA <sup>†</sup>	NA <sup>†</sup>	26.75	28.50	NA <sup>†</sup>	NA <sup>†</sup>
	0.22	42.00	40.25	25.75	26.75	NA <sup>†</sup>	NA <sup>†</sup>	19.25	28.75	NA <sup>†</sup>	NA <sup>†</sup>
	0.44	43.00	41.25	25.50	27.75	NA <sup>†</sup>	NA <sup>†</sup>	26.25	27.50	NA <sup>†</sup>	NA <sup>†</sup>
	2.8	43.00	41.00	25.25	26.75	NA <sup>†</sup>	NA <sup>†</sup>	25.50	28.50	NA <sup>†</sup>	NA <sup>†</sup>
	5.6	43.25	39.50	25.50	27.50	NA <sup>†</sup>	NA <sup>†</sup>	27.25	28.75	NA <sup>†</sup>	NA <sup>†</sup>
	8.4	41.25	39.25	25.50	27.75	NA <sup>†</sup>	NA <sup>†</sup>	28.00	29.25	NA <sup>†</sup>	NA <sup>†</sup>
LSD (0.05)		NS		NS				NS		NS	
2007											
	0	39.00	39.75	31.25	30.50	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	950.13	1018.50
	0.22	37.25	40.00	31.00	31.00	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	951.13	1075.40
	0.44	36.00	37.75	33.00	29.75	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	1020.50	416.40
	2.8	36.00	38.00	31.50	31.00	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	1035.52	790.36
	5.6	30.50	39.50	30.25	30.00	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	526.25	526.25
	8.4	38.50	40.00	32.00	30.50	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	NA <sup>†</sup>	961.25	807.51
LSD (0.05)		NS		NS						NS	

† Data not available

### *Seed Yield*

Treatment effects on soybean yield were not significant at any of the lower yielding (<3000 kg/ha) locations, i.e., Ashland Bottoms 2006 and 2007, Rossville 2006 and 2007, Ottawa 2006, and Manhattan 2007 (Table 2.10 and Table 2.11). Based on yields at Scandia (2005, 2006, and 2007), yield potential was much greater than achieved at these locations. It is likely that variables such as weather, weed pressure, and insects limited yields. Optimum yields are obtained only when favorable environmental conditions are present in all growth stages (Ritchie et al., 1996). Therefore, supplying additional manganese may not have been important with these other yield-limiting factors present. With less variability we may have seen some significant results at Ashland in 2006. For example, increasing foliar Mn application tended to increase GR



soybean yields at Ashland Bottoms but did not seem to affect CV soybean yields. However, this trend was not consistent across locations. For example, trends indicated increased yield at Rossville and Ottawa in 2006 when 0.44 kg Mn/ha was applied to CV soybean and yield did not increase when the same rate was applied to GR soybean. Note that although these treatments seem to show large effects, the effects are not significant.

**Table 2.10.** Analysis of variance (ANOVA) p-values from F-tests for near isoline (GR and CV) and Mn rate (foliar and granular Mn fertilizer) effects on soybean yields.

		Ashland Bottoms	Rossville	Scandia	Ottawa	Manhattan
2006	near isoline	0.456	0.643	<0.001	0.1937	NA <sup>†</sup>
	rate	0.338	0.768	0.001	0.5109	NA <sup>†</sup>
	near isoline*rate	0.066	0.257	<0.001	0.334	NA <sup>†</sup>
2007	near isoline	0.689	0.110	0.795	NA <sup>†</sup>	0.455
	rate	0.483	0.609	<0.001	NA <sup>†</sup>	0.429
	near isoline*rate	0.635	0.322	0.045	NA <sup>†</sup>	0.278

† Data not available

**Table 2.11.** Soybean yield for conventional (CV) and glyphosate-resistant (GR) near isoline at Ashland Bottoms, Rossville, Scandia, Ottawa, and Manhattan in 2006 and 2007.

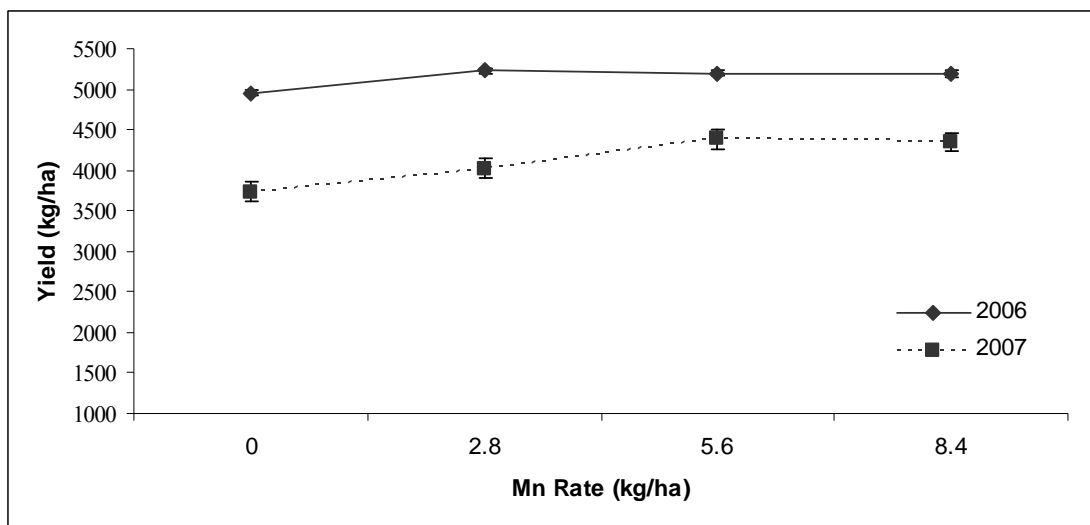
Year	Mn Rate	----- kg/ha -----									
		Ashland Bottoms		Rossville		Scandia		Ottawa		Manhattan	
		CV	GR	CV	GR	CV	GR	CV	GR	CV	GR
2006											
	0	2890	2775	2620	2716	5237	4667	2685	2628	NA <sup>†</sup>	NA <sup>†</sup>
	0.22	2959	3025	2627	2704	NA <sup>†</sup>	NA <sup>†</sup>	2555	2514	NA <sup>†</sup>	NA <sup>†</sup>
	0.44	2991	3112	3118	2589	NA <sup>†</sup>	NA <sup>†</sup>	2702	2376	NA <sup>†</sup>	NA <sup>†</sup>
	2.8	2858	3386	2524	2749	5210	5237	2848	2441	NA <sup>†</sup>	NA <sup>†</sup>
	5.6	2962	2776	2782	2670	5239	5160	2604	2458	NA <sup>†</sup>	NA <sup>†</sup>
	8.4	2797	3046	2666	2652	5093	5290	2677	2734	NA <sup>†</sup>	NA <sup>†</sup>
LSD (0.05)		NS		NS		69		NS			
2007											
	0	2978	2860	2830	2969	3930	3535	NA <sup>†</sup>	NA <sup>†</sup>	1846	1588
	0.22	3102	3136	3213	2993	3976	3958	NA <sup>†</sup>	NA <sup>†</sup>	1701	1718
	0.44	3034	3235	3229	2863	4323	4410	NA <sup>†</sup>	NA <sup>†</sup>	2081	1834
	2.8	3156	3187	3026	2895	4097	3941	NA <sup>†</sup>	NA <sup>†</sup>	1709	1822
	5.6	3071	2805	2993	3074	4253	4514	NA <sup>†</sup>	NA <sup>†</sup>	1941	1409
	8.4	3273	2987	3164	2961	4184	4514	NA <sup>†</sup>	NA <sup>†</sup>	1832	1801
LSD (0.05)		NS		NS		219				NS	

† Data not available

Yields at Scandia were > 3000 kg/ha both years and were significantly affected by rate (2006 and 2007) and near isoline (2006) (Table 2.10). Main near isoline effects at Scandia showed that CV soybean yielded 106 kg/ha more than GR soybean in 2006 when combined across Mn treatments; however, the average CV and GR yields were not significantly different in 2007. These results are similar to findings by Elmore et al. (2001a), where they concluded that CV

soybean yielded 5% more than their GR sister line. Soybean yields reported by Elmore et al. (2001a) were also in excess of 3000 kg/ha.

Main effects for Mn rate were significant in both years at Scandia, where a rate of 2.8 kg Mn/ha would significantly increase yields above the control ( $p>0.0001$ ). However, in 2006 increasing the soil-applied Mn rates above 2.8 kg Mn/ha did not have a significant impact on yield when averaged across varieties (Figure 2.5). Soybean yields at Scandia for the 2007 season not only increased with the 2.8 kg Mn/ha ( $p=0.0121$ ) rate but also significantly increased with a Mn rate of 5.6 kg Mn/ha ( $p=0.0019$ ) when combined across varieties (Figure 2.4).

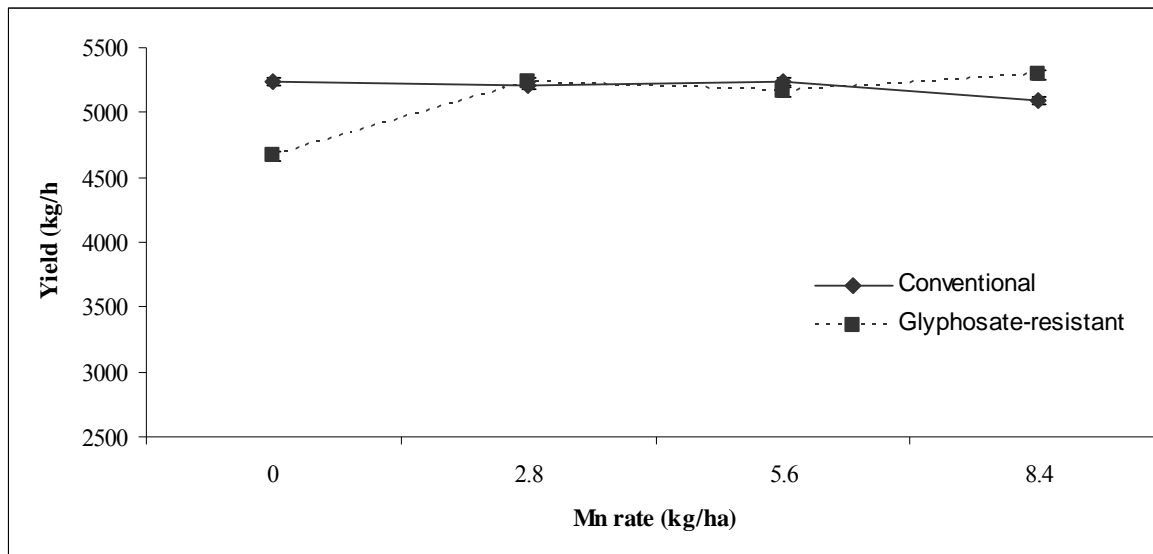


**Figure 2.4.** Rate effect in Scandia for 2006 and 2007 for soil-applied Mn treatments (Error bars are standard error generated by SAS to compare treatments within years).

Results from Scandia are similar to those of Boswell et al. (1981) who found that a rate of 5.6 kg Mn/ha has significant yield benefits for soybean under Mn deficient conditions and the most optimum rate was 11.2 kg Mn/ha. Mascagni & Cox (1985) found that optimum band applications of 3 kg Mn/ha increased soybean yield 14% of the maximum yield. Foliar applications at

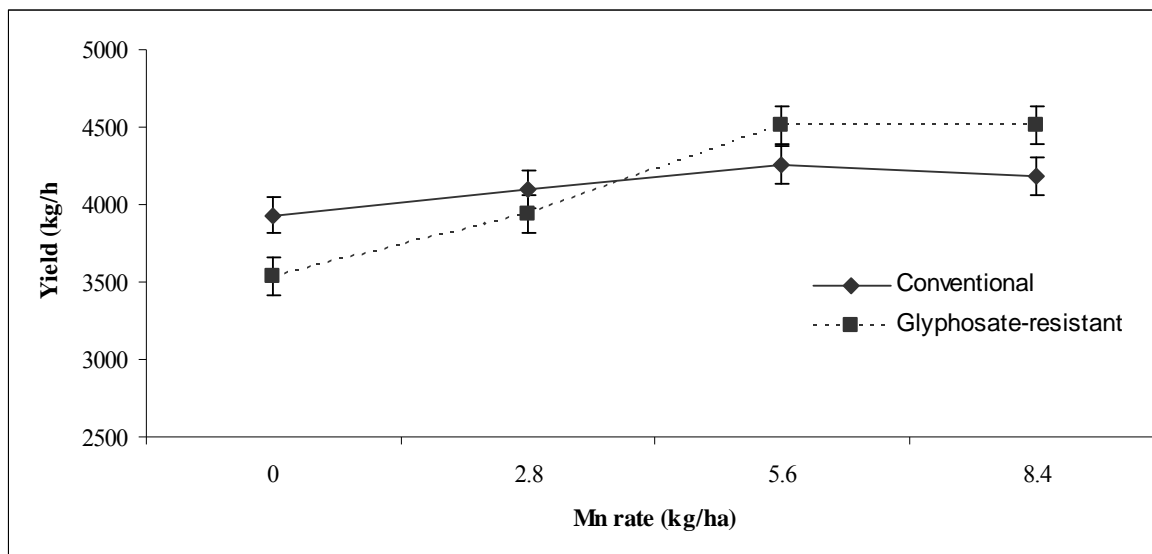
Scandia of 0.44 kg Mn/ha increased yield when combined across near isoline by 634 kg/ha over the control plots (Table 2.11). Mascagni & Cox (1985) also found benefits from foliar application at the 0.1 kg Mn/ha when sprayed once at the first sign of Mn deficiency (V4) but ultimately had their best results with two applications sprayed at both V4 and V10 growth stages.

Results further indicate a significant interaction for near isoline by rate at Scandia in 2006 and 2007 (Table 2.10). At Scandia in 2006, CV soybean yield on average was 569 kg/ha ( $p > 0.0001$ ) greater than GR soybean yield at the 0 kg Mn/ha rate. Although, soil-applied Mn significantly increased yield of GR soybean by 570 kg/ha, soil-applied Mn did not affect CV soybean yields (Figure 2.5). Soybean yields were similar between varieties at Mn rates of 2.8 and 5.6 kg/ha, but CV soybean yields decreased ( $p = 0.0065$ ) when Mn rate increased from 5.6 kg Mn/ha to 8.4 kg Mn/ha.



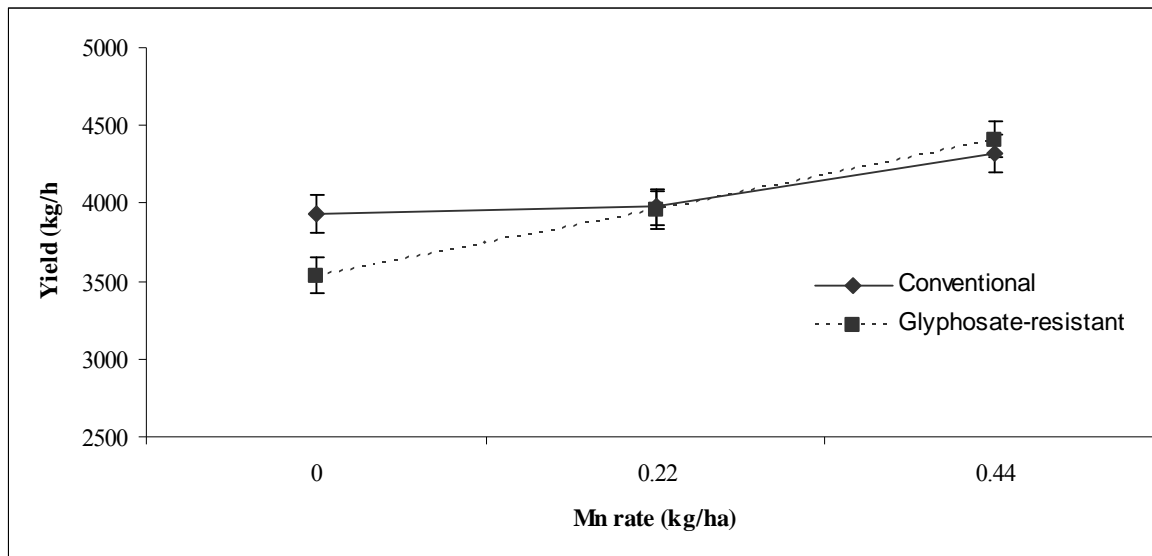
**Figure 2.5.** Scandia location in 2006 soil-applied treatment yields. (Error bars are standard error generated by SAS)

Although GR soybean yields at Scandia in 2007 were less than CV yields at 0 kg Mn/ha, GR soybean yield increased with increasing Mn application up to 5.6 kg Mn/ha (Figure 2.6). Conventional soybean yields also increased with increasing Mn rate, reaching a plateau at 5.6 kg Mn/ha, however, the yield increase from Mn application to CV soybeans was only 323 kg/ha where as Mn application to GR soybean increased yield by 979 kg/ha.



**Figure 2.6.** Scandia location in 2007 soil-applied treatment yields. (Error bars are standard error generated by SAS)

Foliar Mn applications increased yields in both the GR and CV soybean near isoline (Figure 2.7). Glyphosate-resistant yields increased by 432 kg/ha at Mn application of 0.22 kg/ha and yields increased by 874 kg/ha at Mn applications of 0.44 kg/ha compared to the control treatment. Conventional soybean yield increased by 392 kg/ha when applications of 0.44 kg Mn/ha were made, however, foliar-application of 0.22 kg Mn/ha did not significantly increase CV soybean yield. Therefore, GR soybean was more responsive to foliar Mn application than the CV near isoline.



**Figure 2.7.** Scandia location in 2007 foliar treatment yields. (Error bars are standard error generated by SAS)

## Conclusions

Results indicated no yield response to soil-applied Mn treatments in CV or GR soybean at low yielding environments, indicating that Mn was not the most yield limiting factor. At high yielding environments GR soybean in general yielded less than CV near isoline when no Mn was applied, indicating a genetic disposition to yield lag. Also, at high yielding environments GR soybean respond positively to granular Mn treatments when CV soybean yields were not significantly affected with applications of Mn. Treatments as low as 2.8 kg Mn/ha could improve soybean yield, and yields continued to increased with treatments up to the highest rate tested, 8.4 kg Mn/ha.

Foliar Mn applications did not significantly affect yield in low yielding environments. Our results show a large amount of variability in low yielding locations. In high yielding environments foliar treatments of 0.22 kg Mn/ha and 0.44 kg Mn/ha increased GR soybean yield. In addition, rates of 0.44 kg Mn/ha increased yields of CV soybean.

Manganese tissue concentrations in leaf tissue at growth stages R1, R3, and R6 overall were not affected by Mn rate. It is possible that higher rates of Mn may need to be applied to detect differences in concentration. However, at 2 locations a concentration increase was seen with the use of foliar applied Mn. Manganese tissue concentrations did not seem to correlate well with GR soybean yield responses to Mn treatments.

Visual Mn deficiency symptoms were never detected at any of the locations in either year. Also, Mn tissue concentrations or seed concentrations were never low enough to be considered deficient by set standards (Heitholt et al., 2002). These results allude to the fact that Mn deficiency may be much more difficult to accurately detect than previously thought.

### **Future Research**

With significant results indicating a genetic difference for yield in GR soybean compared to CV soybean and a need for Mn fertilization in GR soybean in high yielding environments some applications need further investigations. First, determine if the response to Mn is near isoline specific. Some varieties may respond to Mn fertilizer treatments while other may not be benefited. Next, examine root exudates and enzymatic activity in GR soybean compared to CV varieties. Also, monitoring glyphosate interactions in the rhizosphere with metals, like Mn, could help determine if residual glyphosate entering soils from root exudation inhibits micronutrient

uptake. Finally, development of more accurate testing for plant available Mn may help the implementation of Mn research results.



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# **CHAPTER 3 - Nutrient Concentration, Uptake, and Yield of Glyphosate-resistant and Conventional Soybean**

## **Introduction**

Despite agronomic benefits from glyphosate-resistance in soybean, evidence has shown that the use of glyphosate-resistant soybean varieties can result in reduced yields (Elmore et al., 2001, Gordon, 2005). Results from Chapter 2 show that the glyphosate-resistant (GR) variety K4202RR has reduced yields compared to its near isoline KS4202. In addition, K4202RR responds to Mn fertilization when the conventional (CV) cultivar, KS4202, responds very little or not at all indicating differences in near isoline, possibly due to the glyphosate-resistant genetics. In addition, research by Huber (2006) and Gordon (2005) has shown reduced concentration of Mn in GR soybean. Causes of reduced Mn uptake could also potentially reduce other nutrient concentration and accumulation.

Research suggests a few possible theories as to why GR soybean may need to be managed differently than CV varieties, 1) when inserting the GR transgene into the soybean the genes controlling yield components may be disrupted, 2) the addition of the GR gene is stealing energy or carbon for its protein production rather than being used for seed production, 3) the GR gene may be linked to a gene having a negative impact on soybean yield (Pline-Srnic, 2005), 4) GR gene introduction may alter the root exudates released from the GR soybean plant, thus decreasing nutrient uptake (Kremer et al., 2005), and 5) glyphosate may form insoluble

complexes with some nutrients, thus interfering with nutrient uptake or metabolism (Bernards et al., 2005).

Bertram and Pederson (2004) found that CV soybean and GR soybean managed like CV soybean responded differently to row spacing and seeding rates compared to GR soybean managed with GR weed management strategies. Their research concluded that wider row spacing and increased seeding rates had positive effects on yield in CV soybean and GR soybean managed like CV varieties, however, it did not increase yields for GR soybean managed with GR weed management strategies. Results indicated that GR soybean should be managed similarly to CV soybean, and not with GR soybean management strategies, for example, using the glyphosate herbicide.

Elmore et al. (2001) found that a yield lag of 5% exists in GR soybean varieties compared to their CV soybean sister line. They also found that CV soybean cultivar seed weight was greater and plant height was shorter than the GR soybean near isoline sister line. Although, McCann et al. (2005) analyzed GR soybean over three growing seasons for levels of ash, carbohydrates, moisture, protein, total fat, lectin, trypsin inhibitor, and isoflavones and found that the basic nutritional and biologically active components of GR soybean are compositionally equivalent to that of CV soybean varieties.

Few studies are reported comparing GR soybean production to CV varieties. Even fewer studies have reported results comparing near isogenetic soybean varieties (for GR and CV soybean traits) for nutrient concentration, uptake, accumulation, and yield. Crop mineral composition will

affect yields. Soybean with low or deficient concentration and accumulation of minerals are likely to be more susceptible to plant disease and less tolerant to insect damage (Evans and Thompson, 1979). The objectives of this research were to (a) determine nutrient concentration and uptake in GR and CV soybean at different growth stages and (b) determine differences in yield of soybean near isoline varieties (with and without glyphosate-resistance).

## **Materials and Methods**

In 2005, a field study comparing CV and GR soybean response to Mn fertilization was initiated in Scandia, KS. In 2006 and 2007 the study was extended to Ashland Bottoms, Rossville, Manhattan, and Ottawa, KS (not all locations were used in both years). Soil samples were taken prior to planting and analyzed for P, K, pH, organic matter (OM), Mn, and Fe (Table 3.1). Soil pH was determined by the SMP buffer method (Shoemaker et al., 1961), P concentration was determined by the Mehlich III extraction (Mehlich, 1984), K was extracted with ammonium acetate (Thomas, 1982), Fe and Mn were extracted with the DTPA extraction (Lindsay and Norvell, 1978), and organic matter percent was determined by the Walkley-Black procedure (Walkley, 1947). Primary soil series at the locations are as follows: Rossville: Muscotah silty clay loam (fine, smectic, mesic Aquertic Hapludolls); Ashland Bottoms: Bismarckgrove-Kimo Complex (fine-silty, mixed, superactive, mesic, Fluventic Hapludolls – clayey over loam, smectic, mesic, Fluvenaquentic Hapludolls); Scandia: Crete silt loam (fine, smectic, mesic Pachic Arguistolls); Ottawa: Woodson silt loam (fine, smectic, thermic Abruptic Argiaquolls); and Manhattan: Zeandale silt loam (coarse-silty, mixed, superactive, calcareous, mesic Typic Udifluvents).



**Table 3.1.** Select soil analysis from field locations for 2006 and 2007.

	Location	pH	Om †	Mn	P	K	Fe
2006					----- mg/kg -----		
	Ashland Bottoms	6.5	2.2	23	33	388	44
	Rossville	7.1	1.8	14	16	155	20
	Scandia	6.7	2.7	15	64	438	25
	Ottawa	5.9	2.5	51	30	154	56
2007							
	Ashland Bottoms	6.3	2.9	13	16	194	82
	Rossville	6.3	2.3	31	27	319	42
	Scandia	6.0	2.7	26	8	438	64
	Manhattan	8.3	0.9	4	68	132	9

† organic matter

A randomized, split block design was used to include the Mn rate study reported in Chapter 2 within the near isoline comparison, with near isoline as the whole plot factor and manganese treatment as the sub plot factor. The study had 4 replicates at each location. Plots were 3.1 m wide by 10.7 m long, row spacing was 0.8 m. Soybean varieties were KS 4202 and K4202RR or KS 4602NR and KS 4602NRRR when soybean cyst nematode was a concern or a difficulty of obtaining an isoline (Table 3.2). Seeding rate was approximately 290,000 seeds/ha. The controls plots of 0 kg Mn/ha from the Mn rate study were used for the data in this chapter.

**Table 3.2.** Near isolines of soybean planted at study location in 2006 and 2007.

	Location	Near isolines	Planting Date mm/dd/yyyy
2006	Ashland Bottoms	KS4202 & K4202RR	05.17.2006
	Rossville	KS4602N & K4602NRR	05.16.2006
	Scandia	KS4202 & K4202RR	05.15.2006
	Ottawa	KS4202 & K4202RR	05.22.2006
2007	Ashland Bottoms	KS4202 & K4202RR	05.18.2007
	Rossville	KS4602N & K4602NRR	05.22.2007
	Scandia	KS4202 & K4202RR	05.21.2007
	Manhattan	KS4602N & K4602NRR	06.04.2007

No glyphosate was applied during the growing season but it was used as a pre-plant burn down herbicide at most locations (Table 3.3). Pre-emerge herbicides and manual weeding was used to control weeds during the crop growing season. All locations were irrigated except Ottawa to maintain adequate soil moisture. No additional fertilizers were used except at Rossville and Scandia where a starter fertilizer was used (Table 3.3).

In general, each location had normal to low average monthly precipitation during the growing season compared to 30 year normals. The Ashland Bottoms and Manhattan locations received large rainfall events in August 2006 and May 2007 which brought up monthly averages well above normal. Overall monthly average maximum temperatures were higher than 30 year normals at all locations except Rossville, Ashland Bottoms, and Manhattan which were normal in 2007. Growing season monthly averaged minimum temperatures were cooler than normal at all locations in each year compared to 30 year normal minimum temperatures (Appendix A).

**Table 3.3.** Cultural practices for tillage, herbicide, irrigation, and fertilizer for nutrient study.

Location	Year	Tillage	Herbicide	Irrigation	Fertilizer
Ashland B.	2006	Conventional	None	Furrow	None
Ashland B.	2007	Conventional	Pre-plant: Round-up, Dual, and Authority First	Furrow	None
Rossville	2006	Conventional	Flexstar and Select	Sprinkler	Starter
Rossville	2007	Conventional	Pre-plant: Round-up, Dual, and Authority First	Sprinkler	Starter
Scandia	2006	No-till	Pre-plant: Round-up,	Sprinkler	Starter
Scandia	2007	No-till	Pre-plant: Round-up,	Sprinkler	Starter
Ottawa	2006	No-Till	Flexstar and Select	None	None
Manhattan	2007	Conventional	Pre-plant: Round-up, Dual, and Authority First	Sprinkler	None

Whole plant samples were harvested from 0.58 m<sup>2</sup> (2.3 m total row length over 3 sample periods) of the control plots at approximate growth stages R1, R3, and R6 (except at Scandia in 2006). Plant height and plant number in the plot were recorded at all three growth stages. Plot samples were then separated by pods, stems, and leaves. All partitioned samples were oven dried

at 60° C. Biomass from each plot was prepared for plant digest by grinding to pass a 2 mm sieve with a Wiley Mill grinder. Thirty feet of the center two rows in each plot was harvested at maturity for grain yield using a plot combine. A plot combine was not available at the 2007 Manhattan location, therefore, 20 ft of the center two rows were hand harvested and threshed with an Almaco thresher.

Tissue and grain samples were processed with a nitric acid digest described in the Plant Analysis Handbook II (Mills and Jones, 1991) and by Jones and Case (1990). A 0.50-g sub-sample of each tissue sample was weighed into digestion tubes. Ten ml of concentrated nitric acid was added to each digest tube and allowed to sit over night. Tubes were then placed on a digestion block at 127° C for a 1 hour digest. Twenty ml of 30% hydrogen peroxide was added to each sample and returned to the digestion block for 2 hours at 127° C. Each digestion tube then received 50 ml of 20% HCl and was brought to volume (100 ml) with deionized water. Each digest was then analyzed with an ICP mass spectrometer for N, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, and B concentrations and compared values to sufficient levels (Table 3.4).

**Table 3.4.** Soybean sufficiency levels for N, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, and B at flowering.

Nutrient	Level	References
N	3.25%	Sumner, 1977
P	0.3%	Mills and Jones, 1991
K	1.5%	Peaslee et al., 1985
Mg	0.25%	Parker et al., 1980
Ca	0.8%	Parker et al., 1980
S	0.25%	Hitsuda et al., 2005
Zn	21 mg/kg	Jimenez et al., 1996
Mn	17 mg/kg	Ohki et al, 1979; Mills and Jones, 1991
Cu	4 mg/kg	Mills and Jones, 1991
Fe	25 mg/kg	Mills and Jones, 1991
B	20 mg/kg	Mills and Jones, 1991

Statistical computations were completed with SAS proc mixed models procedures. Each year and location was analyzed separately due to location variations. Repetition was considered a random variable with near isolate being fixed effects (Appendix B). Note that at Scandia in 2006 only plant height and yield data were taken.

## **Results and Discussion**

### ***Nutrient Concentration at Growth Stages R1, R3, and R6***

Overall, very few repeatable differences across years or locations occurred between nutrient concentrations of plant partitions at any of the growth stages sampled (R1, R3, and R6). When differences in concentration did occur they were most often showing the CV soybean cultivar having a higher concentration of a nutrient than the GR variety.

No significant differences were detected between varieties used for N concentration in leaf tissue. Conventional soybean varieties stem nitrogen concentrations were higher at some

locations when compared to the GR variety (Ashland Bottoms 2006: R3 and R6; and Ottawa 2006: R6) and the nitrogen concentration in pods of CV soybean at R6 in Ottawa (2006) was significantly higher than nitrogen concentration in pods of GR soybean (Table 3.5).

No significant differences were detected between varieties used for P concentration in leaf tissue, pods, or seed. Phosphorus concentrations in plant stems of CV soybean were higher compared to that of the GR soybean at Ashland Bottoms 2006 ( $p=0.017$ ) and 2007 ( $p=0.0079$ ) when averaged over sample times (Table 3.6). Phosphorus leaf tissue concentrations were sufficient for plant growth in all locations each year (Mills and Jones, 1991).

No significant concentration differences occurred for near isoline at any location in either year for K (Table 3.7). However, K leaf tissue concentrations at Rossville (2006), Ottawa (2006), and Manhattan (2007) would be considered low at R1 growth stage. In addition, large decreases of K concentration occurred in the leaf tissue at Ashland Bottoms (2006), Scandia (2007), and Ashland Bottoms (2007) for both CV and GR varieties as age of the plant increased. It is important to note that all leaf tissue samples were taken during the reproductive stages (late vegetative stages). Potassium nutrient levels tend to decline during late reproductive stages (Peaslee et al., 1985).

**Table 3.5.** Nitrogen leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- g/kg -----							
		R1	42.8	42.5	16.0	15.5				
		R3	44.0	41.0	14.5***	10.8				
		R6	44.5	45.0	16.0**	13.8	38.0	39.3	55.0	54.8
Ashland B.	2007									
		R1	50.7	46.0	23.3	20.4				
		R3	50.1	62.0	16.0	15.8	82.0	83.4		
		R6	97.0	77.0	17.9	13.1	79.7	83.1	178.4	164.0
Rossville	2006									
		R1	43.3	45.0	20.5	19.3				
		R3	47.0	46.0	16.0	15.8				
		R6	46.5	46.0	16.0	14.3	41.5	38.3	56.8	57.3
Rossville	2007									
		R1	75.9	55.1	22.3	20.5				
		R3	171.6	184.8	54.5	52.2	136.7	167.4		
		R6	170.9	172.3	62.5	81.6	261.6	170.2	100.6	84.1
Ottawa	2006									
		R1	46.3	47.5	16.3	15.8				
		R3	43.5	43.8	14.5**	12.8				
		R6	30.8	32.8	9.5	8.8	33.8**	30.5	60.5	60.3
Scandia	2007									
		R1	58.0	56.1	19.3	17.3				
		R3	55.6	46.7	16.5	15.6	43.4	42.8		
		R6	31.8	46.3	10.7	11.0	41.3	35.3		
Manhattan	2007									
		R1	174.7	182.4	69.6	140.6				
		R3	67.6	171.1	22.2	32.9	22.2	36.9		
		R6	99.3	127.3	25.7	41.3	78.8	132.3	66.6	136.4

‡Seed samples were taken at harvest.

**Table 3.6.** Phosphorus leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- g/kg -----							
		R1	3.5	3.8	3.2	2.8				
		R3	3.0	3.0	3.1	2.5				
		R6	3.0	3.0	2.6	2.2	4.5	3.8	5.8	5.5
Ashland B.	2007									
		R1	3.6	3.3	3.0	2.3				
		R3	3.3	3.1	2.1	1.9	4.3	4.3		
		R6	2.6	2.5	2.2	2.0	4.2	4.2	6.1	5.9
Rossville	2006									
		R1	3.4	3.5	2.7	2.4				
		R3	3.5	3.3	2.7	2.4				
		R6	3.2	2.8	2.5	2.0	4.5	4.1	5.9	5.8
Rossville	2007									
		R1	3.3	3.4	2.2	2.2				
		R3	3.3	3.0	2.2	2.2	4.6	4.7		
		R6	2.3	2.2	1.6	1.4	3.9	3.3	5.9	5.6
Ottawa	2006									
		R1	3.2	3.7	2.2	2.6				
		R3	2.8	2.6	2.1	2.1				
		R6	1.9	2.1	1.4	1.4	3.0	3.1	5.0	5.4
Scandia	2007									
		R1	3.0	2.8	1.8	1.8				
		R3	3.1	3.0	2.2	6.6	4.2	4.2		
		R6	1.4	2.0	0.9	1.2	3.1	2.8		
Manhattan	2007									
		R1	3.3	3.0	2.4	2.3				
		R3	3.0	3.2	2.7	2.7	4.7	4.9		
		R6	2.8	2.3	1.5	2.1	4.0	4.4	6.6	6.2

‡Seed samples were taken at harvest.



**Table 3.7.** Potassium leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
			----- g/kg -----							
Ashland B.	2006	R1	24.3	23.3	32.3	31.3				
		R3	20.8	20.0	32.5	30.5				
		R6	15.8	15.3	18.8	19.3	24.5	20.3	2.1	1.9
Ashland B.	2007	R1	22.1	20.9	35.6	34.4				
		R3	18.3	17.1	31.1	29.1	24.6	26.0		
		R6	15.7	16.7	16.9	17.9	23.5	23.2	2.1	2.0
Rossville	2006	R1	15.8	15.8	20.3	19.3				
		R3	17.3	16.0	22.5	23.0				
		R6	16.5	16.5	16.5	15.5	24.5	22.5	2.1	2.0
Rossville	2007	R1	20.2	21.3	28.0	30.8				
		R3	15.2	15.2	27.7	26.4	25.8	25.9		
		R6	17.3	18.4	13.4	13.5	21.1	20.7	2.0	2.0
Ottawa	2006	R1	9.8	10.0	8.8	9.8				
		R3	11.0	10.5	10.3	10.8				
		R6	7.5	8.0	4.8	6.0	14.8	15.8	1.9	1.9
Scandia	2007	R1	23.6	22.7	39.3	38.5				
		R3	18.3	18.1	31.3	29.6	25.1	26.8		
		R6	15.3	17.5	21.6	24.3	22.0	22.9		
Manhattan	2007	R1	16.9	14.3	27.1	25.1				
		R3	17.7	17.4	24.1	25.7	25.3	25.9		
		R6	17.8	22.0	30.3	30.3	24.9	24.7	2.1	2.0

<sup>‡</sup>Seed samples were taken at harvest.

At Rossville (2007), CV leaf tissue contained higher concentrations of Mg than GR soybean leaves when averaged over sample times ( $p = 0.0007$ ) (Table 3.8). However, in the same year at Scandia, GR soybean leaves had higher concentrations of Mg than the CV soybean counterpart when averaged over sample times ( $p = 0.00442$ ). No significant concentration differences occurred for near isoline at any location in either year for Mg in the stems tissue, pod tissue, and seed tissue.

Ashland (2007) ( $p = 0.025$ ), Rossville (2006) ( $p = 0.042$ ), and Ottawa (2006) ( $p = 0.048$ ) all showed significance for concentration of Ca, with higher levels in CV leaves compared to GR leaves averaged over sample times (Table 3.9). At Rossville (2006) leaf tissue Ca concentrations at R1 and R6 were significantly higher in CV soybean plants. Leaf tissue Ca levels were at high sufficiency at all locations in each year (Parker et al., 1980). No significant concentration differences occurred for near isoline at any location in either year for Ca concentration in pod or seed tissue.

No significant concentration differences occurred in leaf tissue for near isoline at any location in either year for S (Table 3.10). Sulfur concentrations at Ashland (2007) ( $p = 0.001$ ) and Rossville (2006) ( $p = 0.031$ ) were significantly higher in CV stems compared to GR stems when averaged over sample times. In addition, S concentrations in the pods of GR soybeans were significantly greater than for CV soybeans at Manhattan (2007) ( $p = 0.018$ ) when averaged over sample times; furthermore, at Scandia (2007), the leaves of GR soybean had significantly higher concentrations of S than CV leaves at R6. Sulfur levels were sufficient for both varieties for each test year (Hitsuda et al., 2005).

Zinc leaf tissue concentrations were adequate at each test location in both years (Jimenez et al., 1996). No significant Zn concentration differences occurred at any location in either year except at Rossville (2007) stem tissue of GR soybean had higher concentrations of Zn than CV soybean at R1 (Table 3.11).

**Table 3.8.** Magnesium leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
			g/kg							
Ashland B.	2006	R1	4.0	3.8	3.5	3.5				
		R3	3.5	3.5	3.0	2.8				
		R6	3.5	3.8	2.8	2.5	4.0	4.5	2.5	2.5
Ashland B.	2007	R1	4.4	4.5	3.8	3.7				
		R3	3.8	4.0	3.4	3.3	4.5	4.5		
		R6	2.5	2.5	3.1	2.8	3.8	4.0	3.1	3.0
Rossville	2006	R1	6.3	5.8	5.3	4.5				
		R3	4.8	4.5	4.0	4.0				
		R6	3.3	3.1	3.3	3.0	3.9	3.9	2.0	2.3
Rossville	2007	R1	4.6	4.2	3.6	3.7				
		R3	3.5	3.1	3.1	2.6	3.7	3.6		
		R6	1.9	1.6	3.1	2.8	3.3	3.5	2.6	2.5
Ottawa	2006	R1	7.3	6.5	6.8	6.3				
		R3	5.3	4.8	4.3	4.5				
		R6	4.8	4.5	4.3	3.8	2.3	2.5	2.4	2.5
Scandia	2007	R1	4.0	4.2	3.5	3.5				
		R3	3.9	4.0	3.4	3.4	4.4	4.2		
		R6	1.2	2.7	2.3	2.6	3.9	4.2		
Manhattan	2007	R1	5.0	5.0	4.8	5.4				
		R3	4.7	4.7	4.2	4.1	4.7	5.1		
		R6	3.7	3.1	2.5	2.9	4.2	4.3	2.6	2.4

<sup>‡</sup>Seed samples were taken at harvest.

**Table 3.9.** Calcium leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	g/kg							
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- g/kg -----							
		R1	15.8	13.8	15.0	14.5				
		R3	14.8	13.8	13.0	11.8				
		R6	16.3	14.5	10.0	9.5	11.5	12.3	3.5	3.3
Ashland B.	2007									
		R1	15.6	15.1	15.2	14.9				
		R3	14.8	14.2	13.4	12.1	13.5	13.0		
		R6	24.8	21.0	13.2	12.4	8.7	8.8	4.9	4.6
Rossville	2006									
		R1	22.8**	19.5	17.5	15.0				
		R3	17.5	16.5	13.0	13.5				
		R6	17.5*	15.3	10.5	9.8	11.0	10.8	2.8	2.5
Rossville	2007									
		R1	18.1	16.4	11.6	13.8				
		R3	13.5	12.4	13.3	11.0	10.4	11.0		
		R6	22.8	20.3	10.7	10.4	7.5	8.4	3.1	3.0
Ottawa	2006									
		R1	18.0	16.8	15.5	15.5				
		R3	16.8	15.3	12.8	12.8				
		R6	20.3	18.3	12.3	10.3	9.0	9.0	3.3	3.5
Scandia	2007									
		R1	12.6	12.8	13.1	11.9				
		R3	14.7	14.1	13.4	13.5	14.1	12.3		
		R6	11.4	13.6	10.6	9.6	8.6	9.0		
Manhattan	2007									
		R1	15.4	14.7	13.4	15.5				
		R3	16.3	15.8	11.9	12.3	14.3	14.7		
		R6	12.5	10.8	9.1	9.3	11.8	11.5	3.3	3.0

<sup>‡</sup>Seed samples were taken at harvest.

**Table 3.10.** Sulfur leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	g/kg							
			Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		-----							
		R1	2.0	2.0	1.5	1.0				
		R3	2.5	2.0	1.0	1.0				
		R6	2.8	2.3	1.0	1.0	2.0	2.3	3.1	3.0
Ashland B.	2007		-----							
		R1	2.8	2.8	1.8	1.6				
		R3	2.6	2.6	1.3	1.2	2.3	2.3		
		R6	2.1	2.1	1.4	1.2	2.4	2.5	4.2	3.7
Rossville	2006		-----							
		R1	3.0	3.0	2.0	1.5				
		R3	2.8	2.5	1.3	1.0				
		R6	3.0	2.5	1.5	1.0	2.2	2.0	3.0	3.0
Rossville	2007		-----							
		R1	2.8	2.6	1.8	1.8				
		R3	2.7	2.6	1.5	1.4	2.3	2.4		
		R6	1.7	1.8	1.3	1.3	2.5	2.2	3.4	3.1
Ottawa	2006		-----							
		R1	2.1	2.2	1.2	1.1				
		R3	2.2	2.2	0.8	0.9				
		R6	1.7	1.9	1.0	1.1	1.7	1.8	2.9	3.0
Scandia	2007		-----							
		R1	2.8	2.7	1.9	1.7				
		R3	2.3	2.6	1.4	1.3	2.2	2.2		
		R6	1.6	2.4*	1.4	1.6	2.3	2.0		
Manhattan	2007		-----							
		R1	2.4	2.3	1.4	1.7				
		R3	2.0	2.5	1.6	1.9	2.3	2.4		
		R6	2.6	2.4	1.4	1.6	2.2	2.5*	3.5	3.2

‡Seed samples were taken at harvest.

**Table 3.11.** Zinc leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- mg/kg -----							
		R1	43.8	38.3	20.5	20.3				
		R3	42.5	38.0	12.5	10.3				
		R6	35.8	32.5	5.8	5.8	36.3	36.3	46.8	48.5
Ashland B.	2007									
		R1	41.3	37.3	26.0	22.0				
		R3	32.5	34.3	12.5	11.0	40.8	39.0		
		R6	26.0	23.8	8.0	7.3	34.0	32.0	53.0	47.5
Rossville	2006									
		R1	25.0	22.5	16.5	14.5				
		R3	30.5	28.3	9.5	9.3				
		R6	27.5	24.5	5.8	4.0	31.0	28.5	37.0	38.3
Rossville	2007									
		R1	21.8	20.8	10.5	13.3*				
		R3	31.8	28.3	11.8	10.5	40.0	35.8		
		R6	20.3	19.0	6.0	6.0	27.8	23.8	44.0	40.5
Ottawa	2006									
		R1	48.8	42.8	17.8	18.3				
		R3	49.3	43.3	12.3	11.5				
		R6	42.5	36.8	5.8	4.8	28.5	27.5	49.8	48.3
Scandia	2007									
		R1	47.3	43.3	23.8	26.3				
		R3	32.8	33.5	12.8	11.0	39.8	39.5		
		R6	29.5	31.3	7.8	9.0	31.0	27.8		
Manhattan	2007									
		R1	38.6	37.5	17.3	20.0				
		R3	45.0	41.8	13.0	12.3	51.3	54.0		
		R6	39.0	35.0	13.8	22.0	43.5	42.0	52.0	44.8

‡Seed samples were taken at harvest.

Manganese concentrations in leaf tissue at Scandia (2007) ( $p = 0.046$ ) were significantly higher in CV soybean compared to GR soybean when averaged over sample times. In addition, Ashland Bottoms (2007) and Manhattan (2007) had higher concentration of Mn in pod tissue in CV soybean than in GR soybean (Table 3.12). However, at Ottawa (2006) ( $p = 0.007$ ) GR pods had significantly higher Mn concentration than CV pods. Manganese leaf tissue concentrations were sufficient for both varieties during each year (Ohki et al, 1979; Mills and Jones, 1991).

Copper concentrations were significantly higher in CV stems than in GR stems during growth stage R1 at Ashland (2007) and at Scandia (2007) GR stem had greater Cu concentration averaged over sample times ( $p = 0.001$ ). At Rossville (2007) ( $p = 0.017$ ) CV pods had greater Cu concentration than GR pods when averaged over sample times (Table 3.13).

No significant concentration differences occurred in leaf, stems, or seed tissue for near isoline at any location in either year for Fe (Table 3.14). Pods of CV soybeans had significantly higher concentrations of Fe compared to pods of GR soybeans at Ashland Bottoms (2007) ( $p = 0.028$ ) and Scandia (2007) ( $p = 0.048$ ) when averaged over sample times. Iron concentrations were sufficient for soybean growth in the leaf tissue for both varieties (Mills and Jones, 1991)



**Table 3.12.** Manganese leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	mg/kg							
			Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		-----							
		R1	61	59	24	26				
		R3	72	68	25	25				
		R6	84	82	22	24	47	45	30	29
Ashland B.	2007									
		R1	71	70	24	20				
		R3	69	69	17	15	60	51		
		R6	98	99	22	23	38	40	39	40
Rossville	2006									
		R1	85	83	26	23				
		R3	70	64	20	20				
		R6	72	64	18	14	35	36	26	27
Rossville	2007									
		R1	134	98	23	27				
		R3	85	79	21	18	50	44		
		R6	89	81	13	16	31	31	31	31
Ottawa	2006									
		R1	47	47	15	15				
		R3	42	39	11	11				
		R6	50	52	12	13	24	28	27	29
Scandia	2007									
		R1	80	69	24	25				
		R3	67	66	16	15	62	44		
		R6	106	97	30	28	45	52		
Manhattan	2007									
		R1	118	129	36	42				
		R3	102	108	20	20	55	56		
		R6	112	70	26	35	73	58	35	31

‡Seed samples were taken at harvest.

**Table 3.13.** Copper leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
			----- mg/kg -----							
Ashland B.	2006	R1	11.5	9.3	9.0	7.8				
		R3	9.5	10.0	8.0	5.5				
		R6	6.0	5.5	2.3	2.0	7.5*	6.8	15.3	15.3
Ashland B.	2007	R1	12.3	10.0	12.3	9.0				
		R3	10.3	10.3	7.3	6.5	10.3	9.3		
		R6	6.3	5.8	4.0	3.0	10.0	9.5	20.3	16.3
Rossville	2006	R1	11.8	11.0	12.5	12.0				
		R3	10.3	10.5	7.8	6.5	10.5	9.5		
		R6	5.3	5.0	3.3	2.5	9.0	7.8	16.5	17.8
Rossville	2007	R1	11.8	11.0	12.5	12.0				
		R3	10.3	10.5	7.8	6.5	10.5	9.5		
		R6	5.3	5.0	3.3	2.5	9.0	7.8	16.5	15.6
Ottawa	2006	R1	6.0	7.0	4.0	5.5				
		R3	7.5	6.8	5.0	5.0				
		R6	2.3	2.5	1.0	1.0	5.3	5.0	15.3	14.8
Scandia	2007	R1	14.5	13.5	12.0	12.5				
		R3	9.5	9.3	7.0	6.8	10.5	9.5		
		R6	7.8	7.5	4.0	4.0	10.5	8.0		
Manhattan	2007	R1	10.0	10.5	9.6	10.3				
		R3	7.3	8.8	6.3	6.0	8.0	7.3		
		R6	12.3	13.3	10.5	10.8	12.0	12.3	19.0	17.0

‡Seed samples were taken at harvest.

**Table 3.14.** Iron leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
			----- mg/kg -----							
Ashland B.	2006									
		R1	356	379	157	190				
		R3	428	376	219	278				
		R6	172	266	71	83	38	71	102	96
Ashland B.	2007									
		R1	333	304	253	166				
		R3	216	240	79	84	318	218		
		R6	158	154	77	95	62	54	126	164
Rossville	2006									
		R1	349	268	159	122				
		R3	263	244	135	149				
		R6	181	175	52	34	46	37	79	85
Rossville	2007									
		R1	321	357	104	156				
		R3	159	144	73	65	124	101		
		R6	98	80	22	44	68	72	102	102
Ottawa	2006									
		R1	132	109	74	81				
		R3	127	121	51	54				
		R6	75	72	6	3	13	9	84	92
Scandia	2007									
		R1	237	218	94	102				
		R3	282	254	79	96	318	252		
		R6	124	156	45	46	88	82		
Manhattan	2007									
		R1	316	528	206	248				
		R3	148	198	41	49	71	82		
		R6	173	201	111	99	142	126	77	70

‡Seed samples were taken at harvest.

At Rossville (2006) during R1 B concentrations were higher in GR soybean leaf tissue compared to CV soybean leaf tissue (Table 3.15). Conventional soybeans concentrations of B in the stem compared to GR stem partitions were significantly higher at Ashland Bottoms (2007) and Rossville (2007) during R1. At the Manhattan (2007) location pods of GR soybean had higher concentrations of B than CV soybean pods when averaged over sample times ( $p = 0.009$ ). Generally, it is recommended to use a dry ash procedure for B analysis (Wikner, 1986) unless high Ca concentrations are detected in which case a wet digest will provide a sufficient analysis (Mills and Jones, 1991). Ca levels were in large concentrations for soybean leaf tissue throughout the study so wet digest was used for determination of B. Boron concentrations were somewhat high possibly because boron additions can come from scratches in the glassware during the nitric acid digest (Jones and Case, 1990).

**Table 3.15.** Boron leaf tissue, stem, pod and seed concentration at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	mg/kg							
			Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- mg/kg -----							
		R1	46	42	26	25				
		R3	58	54	32	28				
		R6	49	45	23	22	53	50	66	68
Ashland B.	2007									
		R1	37	37	25	25				
		R3	71	59	92*	65	106	86		
		R6	71	67	37	55	55	57	60	52
Rossville	2006									
		R1	43	40*	28	23				
		R3	53	47	27	27				
		R6	51	46	22	20	51	48	85	90
Rossville	2007									
		R1	52	57	31	33				
		R3	56	52	82	64	94	83		
		R6	64	71	28	32	54	67	39	44
Ottawa	2006									
		R1	59	53	30	29				
		R3	58	54	28	28				
		R6	47	45	21	19	1	1	64	64
Scandia	2007									
		R1	61	66	49	46				
		R3	72	69	92	65	112	79		
		R6	71	93	46	39	64	61		
Manhattan	2007									
		R1	113	104	70	72				
		R3	66	71	52	60	65	105		
		R6	70	61	52	48	62	72	39	35

‡Seed samples were taken at harvest.

### *Nutrient Content*

Stem tissue nitrogen content was greater in GR soybean at Ashland Bottoms (2006) when averaged over sample times ( $p = 0.041$ ). Also, stem tissue had greater N content in CV varieties when averaged over sample times at Ashland (2006), however, GR soybean varieties at Rossville (2007) had higher N content than CV. Conventional soybean stem had higher amounts of N than GR soybean stem at Ottawa (2006) during R3. Pods of GR soybean had greater N content than CV soybean pods when averaged over samples times at Rossville (2006) ( $p = 0.002$ ) and Scandia (2007) ( $p = 0.008$ ). Similarly, at Rossville (2007), N content of GR soybean pods at growth stage R6 was greater than CV soybean pods (Table 3.16).

Phosphorus content was greater in the leaves of GR soybean at Rossville (2007) ( $p = 0.0004$ ) and Scandia (2007) ( $p = 0.037$ ) when averaged over sample times (Table 3.17). Glyphosate-resistant pods had a greater P content than the CV cultivar at Scandia, when averaged over sample times, and Rossville (2007) at growth stage R6. Also, soybean seed of CV soybean had greater P content than the GR variety, when averaged over sample times at Manhattan ( $p = 0.033$ ).

Potassium content in GR soybean leaf tissue was greater than in CV cultivar at Rossville (2007) when averaged across sample times ( $p = 0.001$ ); also, at Rossville (2006) GR soybean had greater K content than the CV cultivar at R6. In addition, stems of GR soybean at Rossville (2007) had more K content when averaged over sample times ( $p = 0.028$ ) (Table 3.18). Glyphosate-resistant pod content of K was greater than content in the CV soybean cultivar at Scandia (2007) and Rossville (2006) when averaged over sample times ( $p = 0.003$ ). Also, pod content at Rossville (2007) showed greater K amounts in the GR soybean variety at growth stage

R3. Nitrogen, P, and K are typically mobilized to the seed during pod fill and decreases in plant leaf and stem tissue concentrations and nutrient accumulation will likely occur (Drossopoulos, 1994 and Jimenez et al., 1996). This was prevalent for K and somewhat for P leaves and stems, but N tissue concentrations and accumulation in leaves and stems did not tend to decline with the onset of pod fill.

**Table 3.16.** Nitrogen leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- kg/ha -----							
		R1	31	29	12	10				
		R3	77	60	51	39				
		R6	107	152	73	66	54	66	159	152
Ashland B.	2007									
		R1	51	46	23	20				
		R3	50	62	16	16	82	83		
		R6	97	77	18	13	80	83	178	164
Rossville	2006									
		R1	38	30	13	9				
		R3	67	64	39	32				
		R6	97	147	62	75	41	77**	149	155
Rossville	2007									
		R1	40	72	12	25				
		R3	29	75	22	22	39	141*		
		R6	17	118	8	17	88	100	174	182
Ottawa	2006									
		R1	36	42	12	11				
		R3	85	73	62***	39				
		R6	81	71	39	40	114	91	162	158
Scandia	2007									
		R1	67	171	22	32				
		R3	99	127	25	41	78	132		
		R6	81	140	22	36	66	136		
Manhattan	2007									
		R1	76	55	22	20				
		R3	172	185	55	52	137	167		
		R6	171	172	63	82	262	170	101	84

<sup>‡</sup>Seed samples were taken at harvest.



**Table 3.17.** Phosphorus leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- kg/ha -----							
		R1	2.69	2.42	2.38	1.90				
		R3	5.63	4.41	10.79	9.09				
		R6	7.17	9.47	11.90	10.48	6.28	6.58	15.90	15.19
Ashland B.	2007									
		R1	3.34	2.90	3.03	2.29				
		R3	2.92	3.57	2.01	1.98	7.82	8.66		
		R6	5.99	4.78	3.00	2.62	7.13	7.96	18.04	16.99
Rossville	2006									
		R1	3.02	2.29	1.79	1.1				
		R3	4.95	4.53	6.59	4.93				
		R6	6.36	9.14	9.26	10.67	4.29	8.20**	15.40	15.77
Rossville	2007									
		R1	2.73	5.23	1.57	3.25				
		R3	1.80	4.20	2.85	2.80	4.01	13.74		
		R6	1.07	7.43	1.45	2.50	7.07	8.14	16.63	16.50
Ottawa	2006									
		R1	2.51	3.02	1.56	1.76				
		R3	5.22	4.36	8.8	6.32				
		R6	5.10	4.49	5.48	6.25	10.14	9.23	13.38	14.27
Scandia	2007									
		R1	3.53	8.67	2.05	3.33				
		R3	5.53	8.82	3.60	14.48	7.73	13.27		
		R6	3.65	5.98	1.75	4.52	5.02	11.05		
Manhattan	2007									
		R1	6.09	5.59	3.39	3.05				
		R3	12.75	12.91	11.01	10.40	17.04	19.99		
		R6	8.64	8.47	6.17	7.48	23.49	16.42	12.08*	9.33

<sup>‡</sup>Seed samples were taken at harvest.

**Table 3.18.** Potassium leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- kg/ha -----							
		R1	18.04	15.71	23.47	21.04				
		R3	36.18	28.25	113.18	111.69				
		R6	37.72	54.18	86.17	92.80	34.96	32.26	5.96	5.35
Ashland B.	2007									
		R1	20.16	17.96	36.44	33.76				
		R3	16.43	19.44	29.21	30.53	45.13	52.19		
		R6	35.03	32.20	23.63	25.55	41.09	44.97	6.16	5.72
Rossville	2006									
		R1	13.69	10.46	13.59	8.97				
		R3	23.72	21.65	52.20	45.79				
		R6	33.70	53.67**	61.80	82.24	23.67	45.78**	5.40	5.88
Rossville	2007									
		R1	16.41	33.00	19.56	45.86				
		R3	8.38	20.89	35.35	34.39	21.70	77.23*		
		R6	8.11	60.24	12.29	26.46	37.88	50.72	5.78	5.81
Ottawa	2006									
		R1	7.65	9.06	6.44	6.60				
		R3	21.15	17.77	42.31	33.63				
		R6	19.08	17.64	20.15	26.83	50.58	47.64	5.04	4.99
Scandia	2007									
		R1	27.06	72.61	45.26	71.28				
		R3	32.52	51.47	52.16	78.99	46.42	83.44		
		R6	37.93	53.50	44.00	79.89	36.61	89.90		
Manhattan	2007									
		R1	29.99	26.67	40.00	31.69				
		R3	71.29	70.35	94.03	98.78	89.41	103.87		
		R6	57.75	73.62	122.27	115.51	147.54	90.89	3.87	3.01

‡Seed samples were taken at harvest.

Magnesium content was greater in the leaves of CV soybean at Ottawa (2006) when averaged over sample times ( $p = 0.007$ ); leaf tissue at Rossville (2007) ( $p = 0.0001$ ) and Scandia (2007) ( $p = 0.015$ ) both had greater content when averaged over sample times in the GR soybean varieties (Table 3.19). Also at Scandia during 2007 Mg content was greater in the pods of GR soybean at growth stages R3 and R6. Glyphosate-resistant pod content of Mg at Rossville during 2006 (R6) and during 2007 (R3) was greater than in the CV soybean cultivar whereas at Manhattan (2007) CV soybean content of Mg was greater in the pods at growth stage R6.

The GR soybean variety had greater content of Ca in the leaves at Rossville (2007) at R6, Scandia (2007) ( $p = 0.039$ ) when averaged over sample dates, while at Ottawa (2006) (R3 and R6) CV soybean had greater Ca content in leaf tissue; also at Ottawa greater content occurred in the stem partition for the CV soybean cultivar. Pods of the GR soybean variety at Rossville (2007) at R6, Scandia (2007) ( $p = 0.029$ ) and Rossville (2006) ( $p = 0.002$ ) when averaged over sample times had more content than the GR soybean variety (Table 3.20). Similarly to research by Jimenez et al., (1996) Mg and Ca content tended to decrease with increasing plant age.

Sulfur content in the leaf tissue at Rossville (2007) was greater in the GR variety compared to the CV cultivar at growth stage R6 and at Scandia (2007) ( $p = 0.022$ ) when averaged over sample times (Table 3.21). Glyphosate-resistant stems had greater S content at Ottawa (2006) at growth stage R6. Pod S content at Rossville (2006) ( $p = 0.001$ ) when averaged over sample times and Rossville (2007) at growth stage R3 was greater in GR soybean varieties.

Glyphosate-resistant leaves had greater Zn content at Rossville (2007) ( $p = 0.002$ ) and Scandia (2007) ( $p = 0.041$ ) when averaged over sample times (Table 3.22). Leaf tissue at Ottawa (2006) had more Zn content in the CV soybean at growth stage R3 and R6. Stem tissue in CV soybean at Rossville (2007) had greater Zn content at growth stage R1. Also, at Rossville (2006) ( $p = 0.004$ ) and Scandia (2007) ( $p = 0.011$ ) pods had greater Zn content in the GR variety when averaged over sample times. In addition, GR soybean at Rossville (2007) had greater Zn content in the pods at growth stage R3. Unlike Scandia and Rossville, Zn content in the pod partition at Manhattan (2007) was greater in the CV soybean cultivar than it was in the GR soybean variety ( $p = 0.025$ ). Also, at Manhattan (2007) Zn content was greater in the CV seed when compared to the GR variety ( $p = 0.011$ ).

Manganese content in the leaf tissue and stem tissue at Ottawa (leaf tissue: R6 and stem tissue: R3) was greater in the CV soybean cultivar and at Rossville (2007) Mn content was greater in the GR soybean variety leaf tissue ( $p = 0.003$ ) and stem tissue ( $p = 0.046$ ) when averaged over sample times. In addition, the GR variety had greater content in the pods at Rossville (2006) and Scandia (2007) during growth stage R6 (Table 3.23).

Total Cu content in leaf tissue at Rossville (2007) ( $p = 0.0003$ ) when averaged over sample times was greater in GR soybean than CV soybean; also, Cu content in the stems was higher in the GR soybean cultivar than the CV soybean (Table 3.24). However, at Ottawa the stem of CV soybean had greater content of Cu than GR soybean during growth stage R3. Pod content of Cu was greater in GR soybean at Rossville (2006) and Scandia (2007) ( $p = 0.039$ ) when averaged over sample times and at Rossville (2007) during growth stage R3. Conventional soybean content of

Cu was greater in the pods at Manhattan (2007) during growth stage R6 and in the seed of CV soybean at Ashland Bottoms (2007) and Manhattan (2007).

**Table 3.19.** Magnesium leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- kg/ha -----							
		R1	2.94	2.34	2.55	2.29				
		R3	6.00	4.95	10.58	9.46				
		R6	8.20	11.07	11.59	11.98	5.70	7.78	7.43	7.20
Ashland B.	2007									
		R1	4.04	3.81	3.90	3.68				
		R3	3.39	4.46	3.16	3.43	8.21	9.16		
		R6	5.75	5.01	4.29	3.59	6.59	7.67	9.31	8.65
Rossville	2006									
		R1	5.37	3.66	3.41	2.01				
		R3	6.73	6.25	10.25	8.08				
		R6	6.73	9.65	13.28	15.85	3.80	7.84**	6.02	6.47
Rossville	2007									
		R1	3.74	6.34	2.60	5.51				
		R3	1.91	4.28	4.00	3.33	3.11	10.34*		
		R6	0.85	4.87	2.67	4.59	6.04	8.32	7.35	7.38
Ottawa	2006									
		R1	5.67	5.58	4.69	4.24				
		R3	10.07	8.08	18.00	13.40				
		R6	12.31	9.82	16.67	16.47	13.46	12.26	6.27	6.69
Scandia	2007									
		R1	4.54	12.0	4.02	6.44				
		R3	6.87	11.89	5.59	9.32	8.18	13.02		
		R6	3.03	8.13	4.72	8.67	6.38	16.68		
Manhattan	2007									
		R1	9.09	9.19	6.55	7.90				
		R3	20.16	19.01	16.13	15.29	17.31	20.17		
		R6	11.26	10.91	10.13	10.86	25.30	15.73	4.79	3.77

<sup>‡</sup>Seed samples were taken at harvest.

**Table 3.20.** Calcium leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
			----- kg/ha -----							
Ashland B.	2006									
		R1	11.66	9.10	10.88	9.86				
		R3	25.76	19.83	45.61	42.09				
		R6	39.23	49.68	45.64	46.26	16.37	20.87	10.08	9.75
Ashland B.	2007									
		R1	14.49	12.76	15.79	14.85				
		R3	13.17	16.00	12.57	12.76	24.61	26.00		
		R6	58.70	42.83	17.08	15.68	15.43	16.90	14.53	12.98
Rossville	2006									
		R1	19.59	12.75	11.67	7.13				
		R3	24.64	22.91	32.72	27.32				
		R6	36.02	49.11	40.70	51.21	10.39	21.52**	6.71	7.94
Rossville	2007									
		R1	14.44	25.01	8.46	20.90				
		R3	7.58	17.05	17.09	14.28	9.12	31.79		
		R6	10.53	62.27**	9.22	19.05	13.46	19.86	8.75	8.93
Ottawa	2006									
		R1	14.02	14.88	10.93	10.52				
		R3	33.06	25.72	55.15	39.88				
		R6	52.88	40.17	48.96	45.92	30.21	27.33	8.86	9.37
Scandia	2007									
		R1	14.48	39.79	15.38	23.33				
		R3	26.09	41.92	21.93	36.07	26.21	38.73		
		R6	28.40	41.34	21.92	33.51	14.05	35.82		
Manhattan	2007									
		R1	28.47	27.50	17.82	21.63				
		R3	70.38	63.73	47.59	47.19	53.97	60.72		
		R6	38.37	39.01	36.19	33.97	69.03	41.09	6.08	4.74

<sup>‡</sup>Seed samples were taken at harvest.

**Table 3.21.** Sulfur leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- kg/ha -----							
		R1	1.55	1.45	1.06	.84				
		R3	4.18	3.27	3.65	3.31				
		R6	5.95	8.25	5.84	6.18	3.07	3.68	8.96	8.43
Ashland B.	2007									
		R1	2.59	2.39	1.86	1.56				
		R3	2.33	2.98	1.21	1.24	4.03	4.46		
		R6	4.84	3.89	2.05	1.56	4.11	4.77	12.28	10.59
Rossville	2006									
		R1	2.58	1.84	1.30	0.77				
		R3	3.68	3.46	3.00	2.21				
		R6	5.37	7.71	6.06	6.00	2.20	3.95**	7.98	8.12
Rossville	2007									
		R1	2.20	4.04	1.29	2.70				
		R3	1.48	3.58	1.88	1.77	1.98	6.86		
		R6	0.81	5.91**	1.12	2.17	4.63	5.52	9.48	9.05
Ottawa	2006									
		R1	1.66	1.96	0.81	0.74				
		R3	4.25	3.66	3.46	2.64				
		R6	4.53	4.11	4.00	4.90*	5.85	5.28	7.82	7.96
Scandia	2007									
		R1	3.28	8.05	2.16	3.21				
		R3	4.16	7.46	2.29	3.48	4.03	6.66		
		R6	4.19	7.16	2.85	5.34	3.66	7.47		
Manhattan	2007									
		R1	4.40	4.17	1.97	2.19				
		R3	8.89	10.01	6.46	7.29	8.05	9.48		
		R6	8.39	8.68	5.62	5.87	12.81	9.16	6.54	4.93

<sup>‡</sup>Seed samples were taken at harvest.



**Table 3.22.** Zinc leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
			----- g/ha -----							
Ashland B.	2006									
		R1	31.77	25.77	15.22	13.64				
		R3	73.48	56.36	44.40	37.95				
		R6	86.56	106.99	26.46	27.91	52.79	61.22	134.24	135.23
Ashland B.	2007									
		R1	37.68	32.31	26.92	21.96				
		R3	29.34	38.88	11.69	11.59	74.77	78.60		
		R6	60.17	45.18	11.02	9.57	57.71	59.79	157.52	136.40
Rossville	2006									
		R1	22.53	15.13	11.03	7.10				
		R3	44.30	39.08	23.95	18.87				
		R6	57.06	78.58	21.85	21.27	30.34	58.00**	97.40	104.94
Rossville	2007									
		R1	18.26	32.36	7.56	19.82**				
		R3	17.05	38.85	15.01	13.56	34.15	105.15**		
		R6	9.58	62.97	5.39	10.74	50.27	60.04	125.06	119.99
Ottawa	2006									
		R1	38.60	38.44	12.59	12.51				
		R3	96.36*	73.05	54.04	36.24				
		R6	111.42**	80.30	23.76	21.94	96.85	83.18	132.74	126.45
Scandia	2007									
		R1	55.17	132.97	26.47	48.87				
		R3	58.50	98.66	21.13	29.11	72.74	123.27		
		R6	56.94	93.52	15.87	30.27	50.53	107.93		
Manhattan	2007									
		R1	70.85	65.45	24.64	29.58				
		R3	191.43	162.07	52.49	49.16	188.54	216.92		
		R6	123.74	130.69	55.84	80.85	253.69	152.06	95.50*	67.87

‡Seed samples were taken at harvest.

**Table 3.23.** Manganese leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at p<0.05 (\*), p<0.01 (\*\*), and p<0.0001 (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	g/ha							
			Leaf		Stem		Pod		Seed‡	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- g/ha -----							
		R1	44.26	39.72	17.76	17.68				
		R3	124.66	96.47	88.72	91.06				
		R6	202.96	288.66	101.42	113.95	67.60	75.00	85.76	80.35
Ashland B.	2007									
		R1	65.76	59.00	25.21	19.81				
		R3	61.81	77.80	15.52	15.75	116.38	102.25		
		R6	224.34	205.30	27.14	28.61	64.27	75.37	116.32	113.52
Rossville	2006									
		R1	74.72	52.50	17.52	10.85				
		R3	99.48	90.08	49.43	42.93				
		R6	151.22	201.88	70.59	74.64	35.55	73.62**	68.31	72.14
Rossville	2007									
		R1	112.77	149.52	16.18	40.40				
		R3	45.66	108.70	26.35	22.85	44.44	127.06		
		R6	46.27	243.91	10.45	32.81	56.92	73.77	89.15	90.69
Ottawa	2006									
		R1	36.90	42.20	10.51	10.48				
		R3	80.95	65.34	45.41*	33.28				
		R6	130.98*	112.97	49.24	59.52	81.32	84.95	70.89	75.37
Scandia	2007									
		R1	90.92	204.21	27.44	43.93				
		R3	119.91	189.88	25.80	39.76	112.88	139.05		
		R6	202.39	296.09	61.94	89.92	71.27	205.01**		
Manhattan	2007									
		R1	212.59	246.85	50.04	56.55				
		R3	486.30	446.95	77.72	79.03	204.88	231.42		
		R6	332.73	257.72	104.48	129.06	444.51	215.30	63.12	47.82

‡Seed samples were taken at harvest

**Table 3.24.** Copper leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
			----- g/ha -----							
Ashland B.	2006									
		R1	8.33	6.22	6.52	5.17				
		R3	16.07	15.08	28.66	20.41				
		R6	14.06	19.90	10.32	9.68	10.91	10.86	44.19	42.25
Ashland B.	2007									
		R1	11.39	8.67	12.59	8.79				
		R3	9.18	11.48	6.71	6.87	18.88	18.51		
		R6	15.18	10.17	5.92	4.06	17.71	18.40	60.02*	46.48
Rossville	2006									
		R1	8.06	5.60	6.75	3.99				
		R3	10.65	9.84	13.88	11.82				
		R6	16.07	20.05	18.83	16.09	6.45	12.38*	34.51	35.40
Rossville	2007									
		R1	9.86	16.90	8.76	17.09				
		R3	5.68	14.38	10.01	8.41	8.62	28.12		
		R6	2.41	16.31	2.87	3.90	15.92	19.62	46.98	46.49
Ottawa	2006									
		R1	4.74	6.21	2.70	3.72				
		R3	14.38	11.68	21.27**	15.68				
		R6	5.82	5.47	4.09	4.56	17.84	15.13	41.44	38.92
Scandia	2007									
		R1	16.46	41.48	13.86	23.46				
		R3	17.01	27.99	12.50	18.15	19.48	29.96		
		R6	15.32	22.25	8.14	12.55	17.44	31.41		
Manhattan	2007									
		R1	19.00	19.53	12.80	13.17				
		R3	30.70	34.64	25.04	23.58	29.06	30.04		
		R6	40.06	47.75	42.15	41.64	69.67**	44.45	35.41*	25.59

<sup>‡</sup>Seed samples were taken at harvest.

At Rossville (2007) ( $p = 0.0002$ ) and Scandia (2007) ( $p = 0.042$ ) GR soybean leaves, when averaged over sample times, showed more Fe content than CV leaves (Table 3.25). At Rossville (2007) during growth stage R1 GR soybean had greater Fe content into the stem tissue than CV soybean. Pod tissue at Manhattan (2007) had more Fe content in the CV soybean cultivar than the GR soybean variety ( $p = 0.025$ ).

Ottawa (2006) CV soybean leaf tissue had greater B content than the GR soybean leaf tissue (Table 3.26). However, at Rossville (2007) during growth stage R6 and Scandia (2007) ( $p = 0.007$ ), when averaged over sample times, GR soybean leaf tissue had greater boron content than in the CV soybean leaf tissue. No significant difference between varieties occurred in the pod leaf tissue in any year at any location. More boron content occurred in the CV seed at Manhattan (2007) than in the GR soybean seed.

**Table 3.25.** Iron leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS								
			Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
Ashland B.	2006		----- g/ha -----							
		R1	253.68	252.29	116.44	128.05				
		R3	748.52	594.37	805.07	992.22				
		R6	420.63	944.93	330.76	397.11	53.30	119.7	296.50	268.05
Ashland B.	2007									
		R1	319.75	269.06	271.02	167.63				
		R3	189.76	271.49	74.13	88.43	565.06	386.60		
		R6	339.99	268.99	92.43	112.79	110.03	103.65	371.89	436.57
Rossville	2006									
		R1	322.43	182.93	103.80	58.26				
		R3	365.12	337.01	356.33	319.91				
		R6	377.32	572.87	187.45	178.09	37.51	68.35	205.34	229.69
Rossville	2007									
		R1	256.13	550.63	70.71	241.06				
		R3	88.30	197.47	97.14	82.18	101.85	275.94		
		R6	38.31	259.05	21.80	35.87	120.30	182.79	289.78	304.89
Ottawa	2006									
		R1	105.72	98.94	52.94	55.99				
		R3	247.72	203.22	220.45**	168.10				
		R6	198.01	155.62	25.90	12.84	43.72	26.07	222.61	244.23
Scandia	2007									
		R1	268.58	625.68	133.78	204.76				
		R3	507.62	735.15	137.15	356.81	592.66	793.16		
		R6	241.78	429.54	97.78	152.60	142.09	326.79		
Manhattan	2007									
		R1	572.70	1017.00	266.73	333.06				
		R3	650.21	834.56	160.91	196.79	269.10	334.59		
		R6	566.71	689.30	491.76	389.34	783.65*	467.47	138.70*	106.07

<sup>‡</sup>Seed samples were taken at harvest.

**Table 3.26.** Boron leaf tissue, stem, pod and seed content at growth stages R1, R3, and R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Location	Year	GS	Leaf		Stem		Pod		Seed <sup>‡</sup>	
			CV	GR	CV	GR	CV	GR	CV	GR
			----- g/ha -----							
Ashland B.	2006									
		R1	32.85	28.21	18.59	16.67				
		R3	100.66	78.65	112.46	100.74				
		R6	118.84	150.93	103.67	103.94	76.14	83.67	188.53	189.52
Ashland B.	2007									
		R1	34.17	32.22	25.38	24.59				
		R3	63.98	66.28	85.64	69.36	211.40	186.48		
		R6	139.25	111.40	51.06	78.86	97.78	113.88	178.55	148.04
Rossville	2006									
		R1	31.12	26.97	18.61	10.63				
		R3	76.49	66.01	67.26	55.09				
		R6	104.80	146.21	85.02	105.69	50.32	96.81**	25.25	25.25
Rossville	2007									
		R1	43.46	87.78	22.42	50.11				
		R3	30.35	71.46	106.43	82.65	77.04	265.47		
		R6	24.87	213.06***	23.14	61.93	97.92	151.32	109.71	130.66
Ottawa	2006									
		R1	46.50	47.20	21.33	20.03				
		R3	113.42	90.91	119.64	88.29				
		R6	1221.42	98.14	86.42	87.12	134.09	123.88	173.88	167.75
Scandia	2007									
		R1	65.13	206.70	61.43	87.06				
		R3	126.71	195.96	174.02	172.94	205.41	251.32		
		R6	136.09	265.64	93.61	135.26	106.04	244.77		
Manhattan	2007									
		R1	208.85	183.52	106.92	93.14				
		R3	287.16	285.63	202.88	239.80	235.40	418.43**		
		R6	219.43	229.47	209.87	174.80	368.05	267.17	70.95*	52.80

<sup>‡</sup>Seed samples were taken at harvest.

Concentration and content varied greatly with sample date. Much of the significant differences would be attributed to concentrations building with the age of the plant, translocation of the nutrient, or senescence of plant tissue. Plant sampling prior to reproductive stages could be beneficial in determining differences between varieties so to reduce the variability caused by late season nutrient changes.

### ***Total Nutrient Uptake***

Nutrient uptake was determined by summing the nutrient content of plant leaves, stems, and pods at growth stage R6. Nitrogen, P, K, Ca, S, Zn, Mn, and B uptake was greater in GR soybean at Rossville during 2006 (Table 3.27 and Table 3.28). Similarly, N, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, and B uptake was greater in GR soybean variety compared to the CV soybean cultivar at Scandia and Rossville during 2007. Greater nutrient uptake by GR soybean varieties at Rossville during 2006 and 2007 and Scandia during 2007 can likely be attributed to greater plant biomass of GR soybean varieties (Appendix D). At Manhattan, the CV soybean cultivar had greater uptake of K, Mg, Ca, Zn, and Mn.

**Table 3.27.** N, P, and K accumulation (values are for leaves, stems, and pods) during growth stage R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Year	Location	N		P		K	
		CV	GR	CV	GR	CV	GR
2006		----- kg/ha -----					
	Ashland B.	235	285	25	27	159	179
	Rossville	200	299*	19	28**	119	182**
	Ottawa	235	203	21	20	90	92
2007	Ashland B.	195	174	16	15	98	102
	Rossville	115	237*	9	18*	58	137**
	Scandia	171	314**	10	22*	118	223***
	Manhattan	496	425	38	32	327**	280

**Table 3.28.** Ca, Mg, and S accumulation (values are for leaves, stems, and pods) during growth stage R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Year	Location	Ca		Mg		S	
		CV	GR	CV	GR	CV	GR
2006		----- kg/ha -----					
	Ashland B.	26	31	101	117	15	18
	Rossville	3.5	3.2	87	121*	14	18*
	Ottawa	42	39	132*	113	14	14
2007	Ashland B.	24	33	91	75	11	10
	Rossville	10	18**	33	102**	7	14*
	Scandia	14	34**	64	110**	11	20*
	Manhattan	47*	38	144*	114	27	24



**Table 3.29.** Zn, Mn, Cu, Fe, and B accumulation (values are for leaves, stems, and pods) during growth stage R6 for conventional (CV) and glyphosate-resistant (GR) soybean for each test location. Asterisks denote significance at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.0001$  (\*\*\*) for comparisons between varieties within growth stage, plant part, location, and year.

Year	Location	Zn		Mn		Cu		Fe		B	
		CV	GR	CV	GR	CV	GR	CV	GR	CV	GR
----- g/ha -----											
2006	Ashland B.	166	196	372	478	36	41	804	1462	299	338
	Rossville	109	158**	257	350*	42	49	602	819	240	348*
	Ottawa	232*	185	262	257	28	25	268	195	342	309
2007	Ashland B.	129	115	315	309	39	33	542	485	288	304
	Rossville	65	134*	113	350*	21	40*	180	477*	146	426**
	Scandia	123	231**	335	591**	41	67**	482	909**	335	645**
	Manhattan	433*	363	881*	602	152	134	1842	1546	797	671

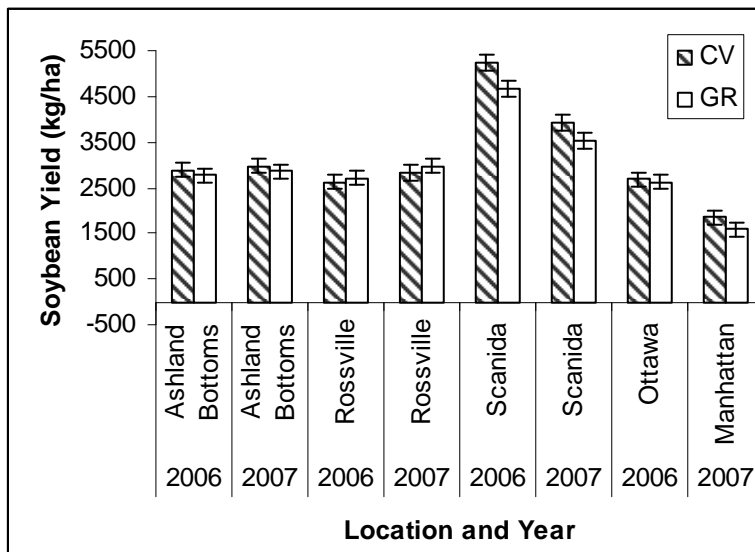
The Manhattan location had the least desirable growing conditions with a planting depth too deep, very sandy soil, and high soil pH. In addition, irrigation at the Manhattan location was difficult to manage. All of these variables contributed to plant stunting. With biomass being similar between varieties (at Manhattan) and CV soybean tending to have higher nutrient concentrations, more nutrient uptake occurred in CV soybean. Zn and Ca accumulation at Ottawa was greater in the CV soybean. Like at the Manhattan location water was an issue at Ottawa where no irrigation was available so some dry periods did exist during the growing season possibly retarding GR plant biomass (Appendix D).

### *Yield*

At all lower yielding (<3000 kg/ha) location; Ashland Bottoms and Rossville during 2006 and 2007, Ottawa during 2006, and Manhattan during 2007, no significant yield differences were detected when comparing varieties. Knowing that yield potential much greater than achieved,

based on yields at the Scandia location, at these locations it is evidence that variables like weather, weed pressure, and insects limited yields so that an accurate assessment of near isoline could not be made. Optimum yields are obtained only when favorable environmental conditions are present in all growth stages (Ritchie et al., 1996). Trends appeared to show higher yielding CV soybean at all location in each year except for Rossville during 2006 and 2007 (Figure 3.1). In addition, yields at Scandia during 2006 and 2007 were considered higher yielding (> 3000 kg/ha) and during 2006 CV soybean yielded 569 kg/ha significantly higher than GR soybean ( $p > 0.01$ ) (Table 3.30).

**Figure 3.1.** Soybean yield in kg/ha for CV and GR soybean varieties (error bars are standard error generated by SAS).



**Table 3.30.** Analysis of variance (ANOVA) p-values from multiple comparisons for near isoline (GR and CV) yield at the 0 mg Mn/ha rate.

Year	Effect	Ashland B.	Rossville	Scandia	Ottawa	Manhattan
2006	near isoline	0.589	0.553	<0.0001	0.343	
2007	near isoline	0.649	0.432	0.014	Abandoned	0.365

## Conclusions

In general, the CV cultivars used in this research tended to have equal to or higher concentrations of N, P, Ca, S, Cu, and Fe during either of the growth stages sampled (R1, R3, and R6) for any of the plant partitions except the seed. The concentrations differences were not usually repeated across locations or years. Plant seed N, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, and B concentrations were never significantly different when comparing near isoline. Potassium and Zn did not appear to be affected by the addition of the GR gene or present growing conditions.

Nutrient content differences most often occurred in plant stems. In soybean near isonlines, KS4202, K4202RR, KS4602N, and KS4602NR, seed nutrient content differences for N, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, and B were not detected in the soybean seed except at Manhattan in CV soybean seed had greater P, Zn, Cu, Fe, and B content than the GR soybean seed. When increased nutrient content occurred it was generally due to increased plant biomass (Appendix D).

Overall, nutrient uptake was significantly greater in GR soybean when growing conditions were optimal and stands were excellent. Under drought stress, weed pressure, and insect pressure CV soybean cultivars tended to take up equal or more nutrients than GR soybean varieties, like at Ashland in 2006 and 2007.

In optimum growing conditions a yield drag does exist for the GR soybean variety K4202RR

compared to the CV soybean cultivar KS4202. All practices were very similar for each near isoline indicating differences for the varieties, providing evidence that more research is necessary to determine if the yield drag is due to the GR gene and what if any additional management practices are necessary to eliminate yield drag from the GR gene.

### **Future Research**

There is enough research completed to suggest that nutrient concentration in CV soybean is sometimes higher than GR soybean, while nutrient content tends to be greater in GR soybean. However, more soybean isoline comparisons are needed to verify if nutrient concentration and is significantly higher in CV soybean plants compared to their GR counterpart and if there are differences are they due to glyphosate-resistant genetics? In addition to further isoline sampling, more early stage plant sampling could help provide information on early season nutrient concentration, accumulation, and fertilizer management strategies.

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# **CHAPTER 4 - Glyphosate Application Effects on Mn Uptake and Accumulation in Glyphosate-resistant Soybean with Manganese**

## **Fertilization**

### **Introduction**

Previous field research has shown that glyphosate-resistant (GR) soybean may respond differently to Mn fertilizer applications than conventional (CV) soybean (Chapter 2). Also, Huber (2006) and Gordon (2005) found that GR soybean responded to Mn fertilizer applications by having increased Mn concentrations in soybean tissue and yield. The CV varieties tested responded very little or not at all to Mn fertilizer applications. In addition to genetic effects on Mn uptake and Mn response in soybean, it is possible that glyphosate application to GR soybean may also interfere with Mn uptake, translocation, or metabolism.

A large amount of glyphosate applied to GR soybean reaches the top 15 cm of soil through direct contact, wash from leaves, and root exudates (Eker et al., 2006). Glyphosate half-life in soil is generally moderate (a few days), but can be very long, lasting weeks to several years (Cerqueira and Duke, 2006). In addition, glyphosate moves quickly in plant tissue and is translocated to metabolic sinks like nodules, seeds, and roots where the glyphosate can then be lost from the system through root exudates (Kremer, 2005).



It has been suggested that glyphosate interacts with micronutrients in the plant tissue and the soil rhizosphere to create mineral complexes potentially inducing nutrient deficiencies (Bernards et al., 2005). Glyphosate causes a reduction in phytoalexins and increases amino acids also released in root exudates (Kremer et al., 2005). In addition, the ratio of Mn-reducing to Mn-oxidizing bacteria has been found to be lower under GR soybean when no glyphosate is applied and the ratio is even lower with glyphosate applications, indicating some sort of interaction in the soil solution with glyphosate and Mn (Kremer, 2008).

Bernards et al. (2005) found that Mn sulfate with lignin sulfonate chelate, Mn sulfate monohydrate, and Mn sulfate with ethylaminoacetate chelate all reduced glyphosate efficacy in field and greenhouse research. They hypothesized that the reduced glyphosate efficacy was a result of Mn-glyphosate complexes formed on the leaf surface or in the plant tissue. They also reported that Mn ethylenediaminetetraacetate (Mn-EDTA) did not result in decreased efficacy of glyphosate. Furthermore, research by Bernard et al. (2005) has shown that Mn additions to glyphosate, especially Mn chelate, significantly reduced the acidifying effect of glyphosate which is critical to its performance. The reduction in the acidifying effect is due to the glyphosate-Mn interactions (Bailey et al., 2002).

Glyphosate used as a pre-emergence burn down herbicide is exuded from target plants, stabilizing, and altering interactions in the rhizosphere which can negatively affect non-target plants (Neumann et al., 2006). Eker et al. (2006) found that low rates of glyphosate to sunflower, a non-target plant, had significant reductions of Fe and Mn concentrations in leaf tissue and reduced translocation within the plant. Also, research has shown that glyphosate disturbs ferric

reductase activity in sunflower roots, but Mn metabolism was not monitored (Ozturk et al., 2008).

Elmore et al. (2001) found that glyphosate-resistant varieties have a yield lag compared to their conventional isoline. Antagonistic effects of glyphosate on nutrient uptake may contribute to this yield lag (Bernards et al., 2005); however, more information is needed on glyphosate effects on nutrient uptake in glyphosate-resistant soybeans to confirm or refute this hypothesis. Most research on the antagonistic effects of glyphosate mixed with Mn has evaluated the efficacy of glyphosate for weed control and not that of the GR soybean ability to utilize the Mn fertilizer. The objectives of this research were to determine if glyphosate has a negative impact on Mn uptake in glyphosate-resistant soybean varieties by: (a) quantifying Mn uptake when glyphosate is and is not applied to glyphosate-resistant soybean; and (b) determine glyphosate effect on soybean response to foliar- and soil-applied Mn treatments.

## Materials and Methods

A greenhouse study was initiated at Kansas State University, Manhattan, KS in 2006. The experiment was a 2-way factorial arranged in five randomized complete blocks with glyphosate application and manganese treatment as the factors. Glyphosate-resistant soybean variety K4202RR was selected to compliment current field research. Standard #2 pots (23 cm deep and 25 cm dia.) were filled with 6.8 kg of soil, where each pot represented an experimental unit. Soil mapped as a Bismarkgrove-Kimo Complex (fine-silty, mixed, superactive, mesic, Fluventic Hapludolls – clayey over loam, smectic, mesic, Fluvenaquentic Hapludolls), was collected from the Ashland Bottoms experiment station, and autoclaved at 121 degrees C for 2 hours. The soil was analyzed for pH, organic matter, Mn, P, K, and Fe (Table 4.1).

**Table 4.1.** Soils analysis from the Ashland Bottoms location used for the soybean greenhouse study.

Soil Series	pH	om <sup>†</sup>	Mn	P	K	Fe
		%	----- mg/kg -----			
Bismarkgrove-Kimo Complex	6.5	2.2	23	33	388	44

<sup>†</sup>organic matter

Greenhouse photoperiod was set at 17 hours of daylight supplemented with sodium high pressure lights. Temperatures were maintained approximately at 24 °C. Manganese treatments included soil-applied MnSO<sub>4</sub> at 0, 8.5, 17, and 25.5 mg Mn/pot and foliar applications of 0.66 and 1.33 mg Mn/pot (Mn chelate MnEDTA, Claw EL). The MnSO<sub>4</sub> was dissolved in DI water and placed in the center of the pot 2.54 cm below the soil surface at planting for more uniform application. Four seeds were then placed at 2.54 cm below the soil surface around the center of the pot. Glyphosate (Roundup WeatherMAX) rates were equivalent to 0 kg/ha and 1.12 kg/ha and were tank mixed with conditioners; AMS, UAN, and foliar Mn treatments.

Soybeans were planted September 12, 2006, emerged 3 days after planting (DAP), and were thinned to one plant per pot at 17 DAP. At growth stage V6 (48 DAP), foliar Mn treatments and glyphosate applications were made with a spray booth. Above-ground biomass was harvested at the soil surface at growth stage R2 (78 DAP), dried at 60 °C, and prepared for digestion by grinding to pass a 2 mm sieve with a Wiley mill.

Plant samples were processed with a nitric acid digest described in the Plant Analysis Handbook II (Mills and Jones, 1991) and by Jones and Case (1990). A 0.50-g plant tissue sample from each plot was weighed into digestion tubes. Ten ml of concentrated nitric acid was added to each digest tube and allowed to sit over night. Tubes were then placed on a digestion block at 127 degrees C for a 1-hour digest. Twenty ml of 30% hydrogen peroxide was added to each sample and samples were returned to the digestion block for 2 hours at 127 degrees C. Each digestion tube then received 50 ml of 20% HCl and was brought to volume (100 ml) with deionized water.

Manganese concentrations of digests were determined with an ICP mass spectrometer. Data were processed with SAS proc GLM procedures. Soil- and foliar-applied Mn treatments were analyzed separately for Mn concentration in plant tissue and total Mn accumulation per plant (or plot) (Table 4.2).

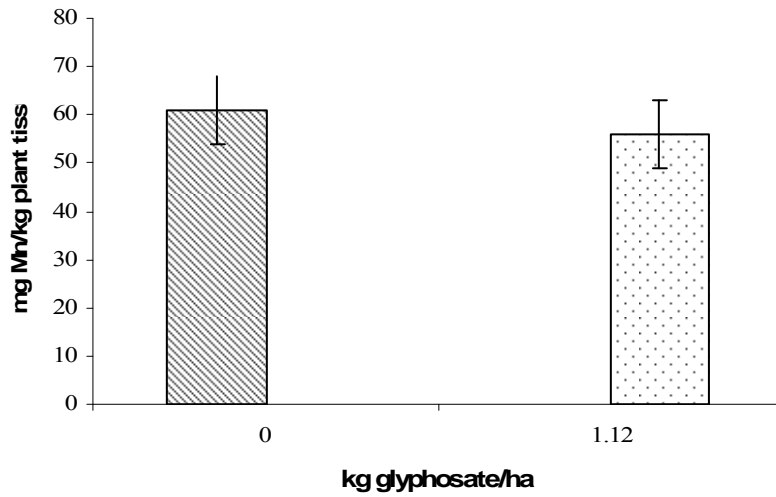
**Table 4.2.** SAS GLM procedure with degrees of freedom and error term used for the F-test.

Source	Degrees of freedom		
	Method of calculation	Value	Error term used for F-test
Rep	rep-1	4	SSE/(44-15)
glyphosate	glyphosate-1	1	SSE/(44-15)
Rate	rate-1	5	SSE/(44-15)
glyphosate*rate	(glyphosate-1)(rate-1)	5	SSE/(44-15)

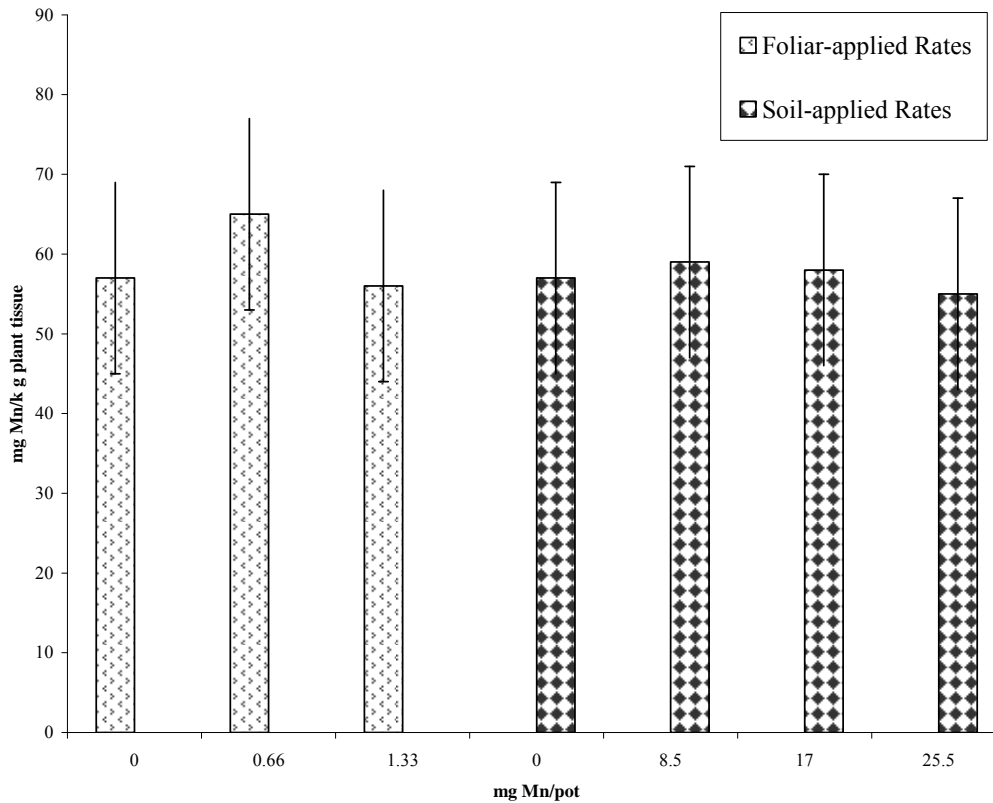
## **Results and Discussion**

### ***Manganese Tissue Concentration***

No significant differences in tissue concentration for Mn were seen with a comparison of glyphosate applications ( $p=0.19$ ) (Figure 4.1). Furthermore, Mn fertilizer rate had no impact on Mn tissue concentrations for the soil or foliar-applied treatment ( $p=0.58$ ) (Figure 4.2) and there was not a significant interaction between glyphosate application and Mn fertilizer rate for Mn tissue concentration (Table 4.3, Figure 4.3, & Figure 4.4). Critical values for manganese concentration in leaf tissue are between 16 to 22 mg Mn/kg dry plant matter (Ohki et al, 1979; Mills and Jones, 1991).



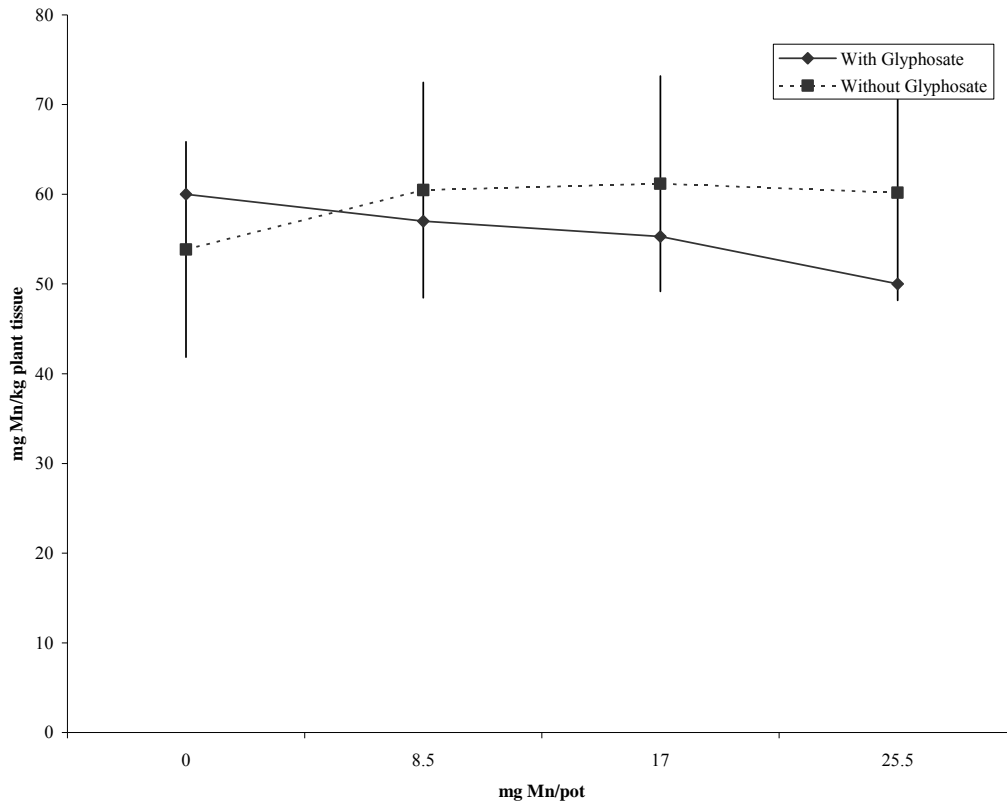
**Figure 4.1.** Manganese tissue concentrations at growth stage R2 in soybean biomass with soil-applied and foliar Mn treatments, averaged across all Mn treatments when glyphosate was and was not applied (error bars are standard error generated by SAS).



**Figure 4.2.** Manganese tissue concentrations at growth stage R2 in soybean biomass with soil-applied and foliar Mn treatments, averaged across glyphosate treatments (error bars are standard error generated by SAS).

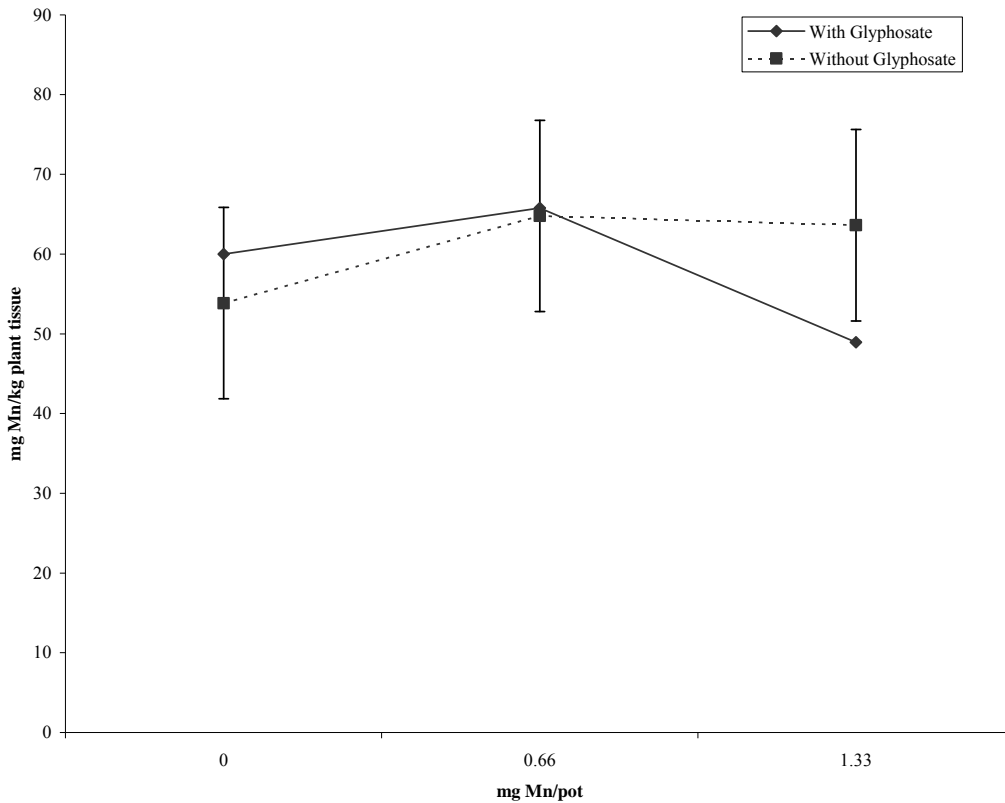
**Table 4.3.** *P*-values for the F-tests from ANOVA for soybean tissue Mn concentrations at growth stage R2.

Source	p-value
Rep	0.62
Glyphosate	0.19
Rate	0.58
glyphosate*rate	0.54



**Figure 4.3.** Manganese tissue concentrations at growth stage R2 in soybean biomass with soil-applied Mn treatments, with and without glyphosate application (error bars are standard error generated by SAS).





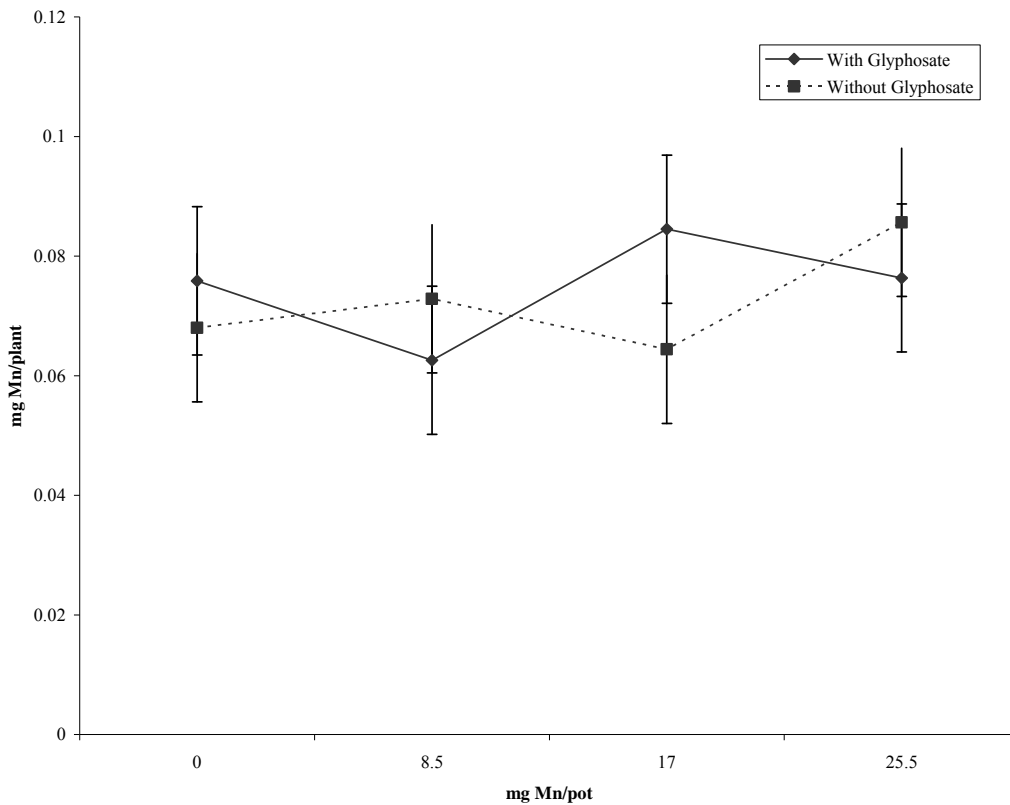
**Figure 4.4.** Manganese tissue concentrations at growth stage R2 in soybean biomass with foliar-applied Mn treatments, with and without glyphosate application (error bars are standard error generated by SAS).

### *Manganese Tissue Accumulation*

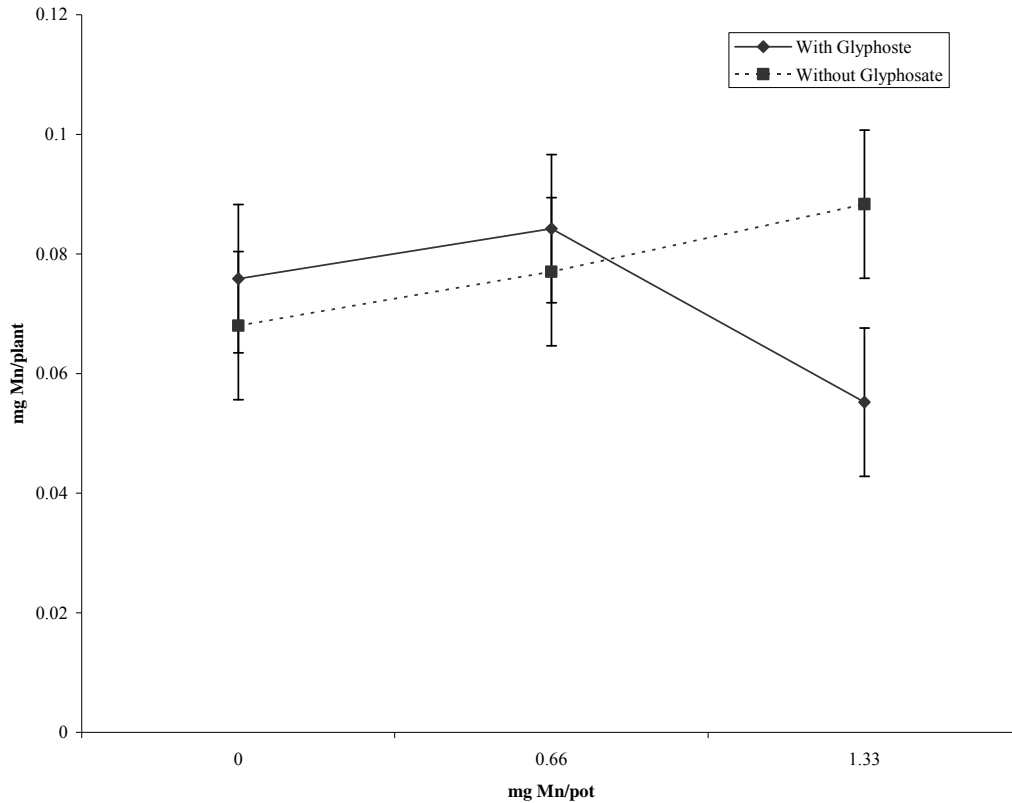
Total Mn uptake was not affected by glyphosate application in the soil or foliar-applied plots (Table 4.4). Furthermore, Mn treatment did not significantly affect Mn uptake for the soil or foliar-applied plots ( $p=0.87$ ) (Figure 4.5 & Figure 4.6). Plant growth, as measured by total biomass at the end of the experiment, was not significantly affected by either glyphosate application or Mn rate ( $p>0.05$ ).

**Table 4.4.** *P*-values for the F-tests from ANOVA for Mn uptake by soybean at growth stage R2.

Source	p-value
Rep	0.45
Glyphosate	0.68
Rate	0.87
glyphosate*rate	0.35



**Figure 4.5.** Manganese accumulation in above-ground biomass at growth stage R2 in soybean with soil-applied Mn treatments, with and without glyphosate application (error bars are standard error generated by SAS).



**Figure 4.6.** Manganese accumulation in above-ground biomass at growth stage R2 in soybean with foliar-applied Mn treatments, with and without glyphosate application (error bars are standard error generated by SAS).

Bailey et al. (2002) found that glyphosate inhibited Mn uptake at various rates of Mn application. Also, when glyphosate was not applied increasing rates of Mn caused an increase in Mn concentration in plant tissue. These results seem contrary to ours because we found no increases in Mn concentration of soybean tissue. In addition, no Mn accumulation increases occurred with increased Mn rates when glyphosate was not applied versus those when glyphosate was applied. If in fact glyphosate and Mn form insoluble complexes which reduce the efficacy of glyphosate we could also expect reduced Mn concentration and accumulation which did not occur in our research.

The results of this study did have a high amount of variability which could have contributed to the lack of significant results. The coefficient of variation for Mn concentration was 22% and for Mn accumulation was 37%. However, the CV for accumulation is not very useful due to the sample mean being near 0 (Wikipedia, 2008). For Mn concentration a study with 21 reps would be required to detect a difference of 10 mg Mn/kg (Steel et al., 1997).

## **Conclusions**

In this study the application of glyphosate had no impact on the Mn tissue concentration or total Mn uptake in soybean. Plant biomass was not altered by the application of glyphosate and glyphosate did not induce a Mn deficient environment. Manganese fertilizer rate did not alter the concentration of Mn or total uptake in soybean plant biomass. It is likely that even if glyphosate had cause Mn to become less available in the soil that there was still enough available Mn to supply the plant demand (i.e. Mn soil test levels of 33 mg Mn/kg soil).

## **Future Research**

Although glyphosate did not induce a deficiency for Mn in soybean plant tissue no variables were tested to determine if Mn became less available in the soil rhizosphere. It may be possible to see significant results in Mn deficient soil conditions.

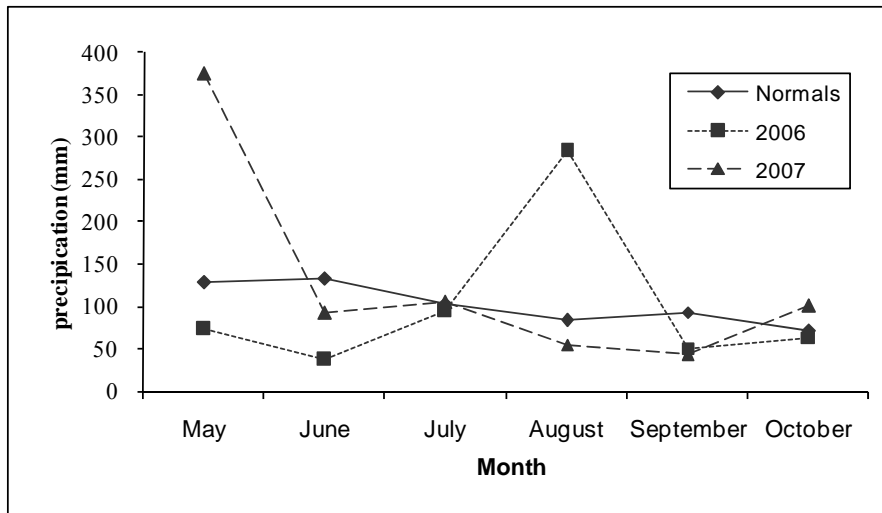
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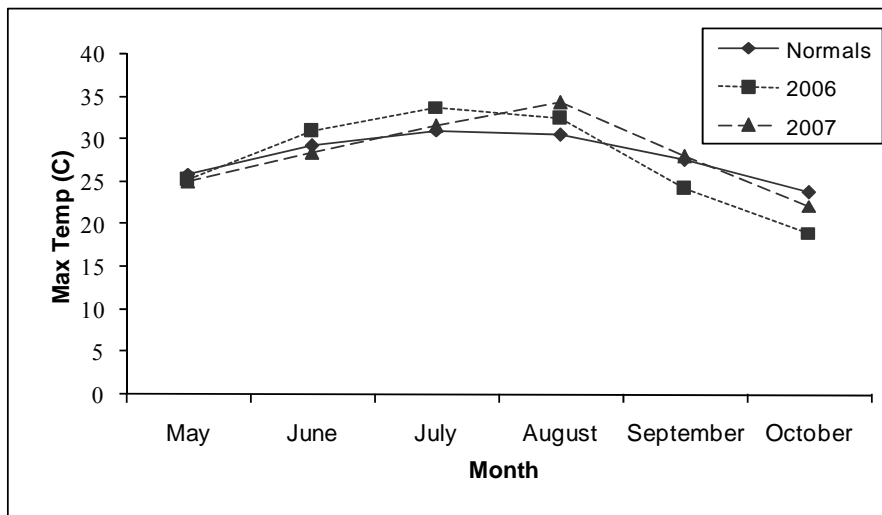
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## Appendix A - Weather Data

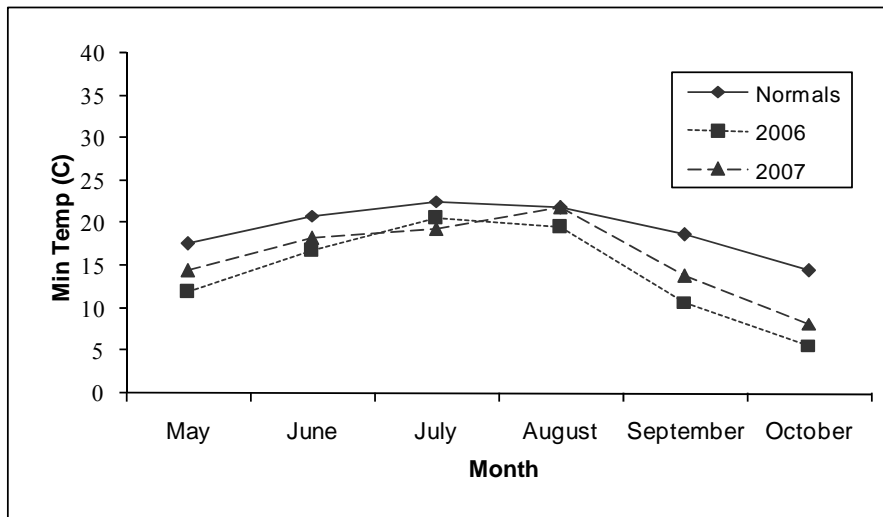
### *Ashland Bottoms/Manhattan Location*



**Figure A.1.** Growing season precipitation in mm for 30 year normals, 2006, and 2007 at the Ashland Bottoms and Manhattan locations.

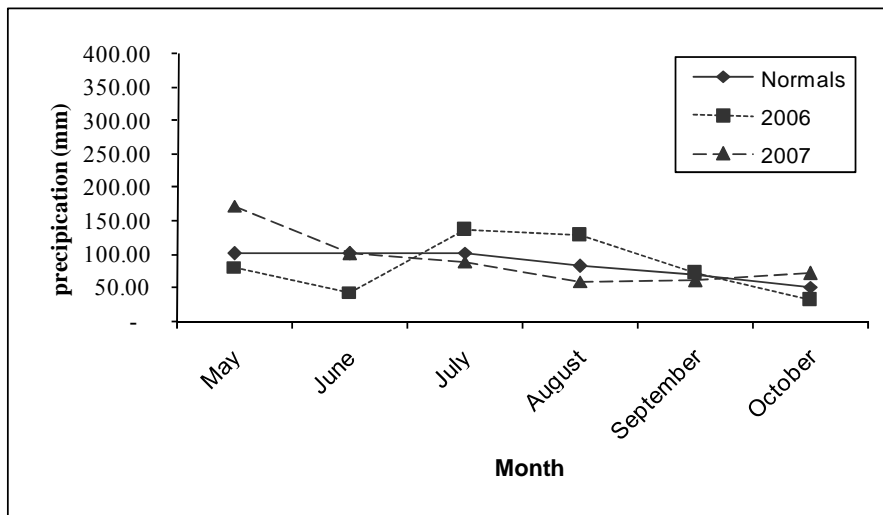


**Figure A.2.** Growing season maximum temperatures in °C for 30 year normals, 2006, and 2007 at the Ashland Bottoms and Manhattan locations.



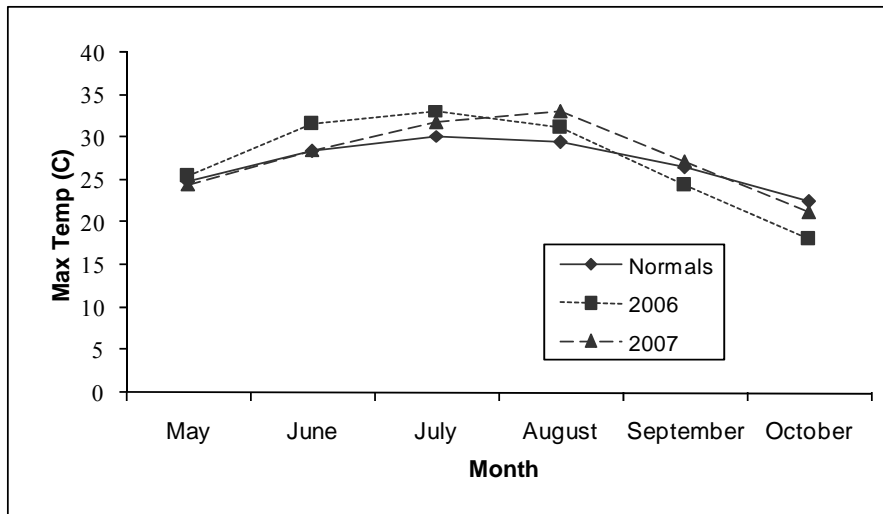
**Figure A.3.** Growing season minimum temperatures in °C for 30 year normals, 2006, and 2007 at the Ashland Bottoms and Manhattan locations.

***Scandia Location***

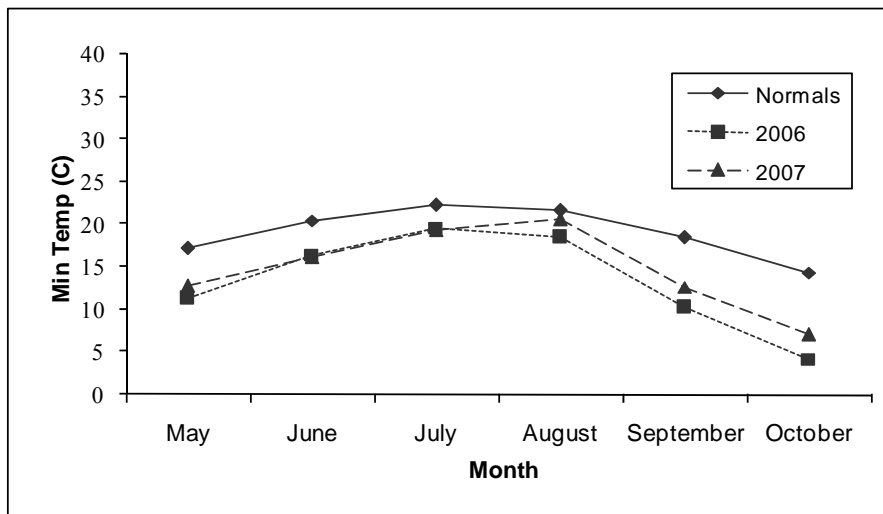


**Figure A.4.** Growing season precipitation in mm for 30 year normals, 2006, and 2007 at the Scandia location.



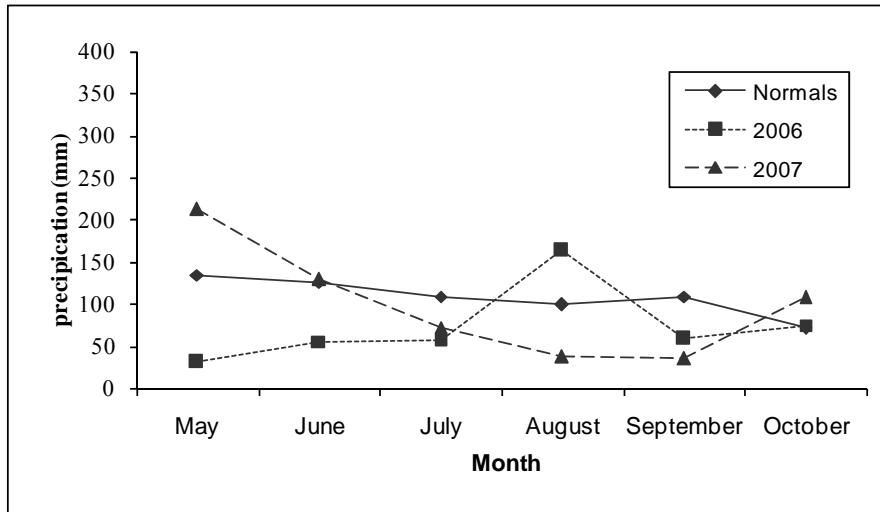


**Figure A.5.** Growing season maximum temperatures in °C for 30 year normals, 2006, and 2007 at the Scandia location.

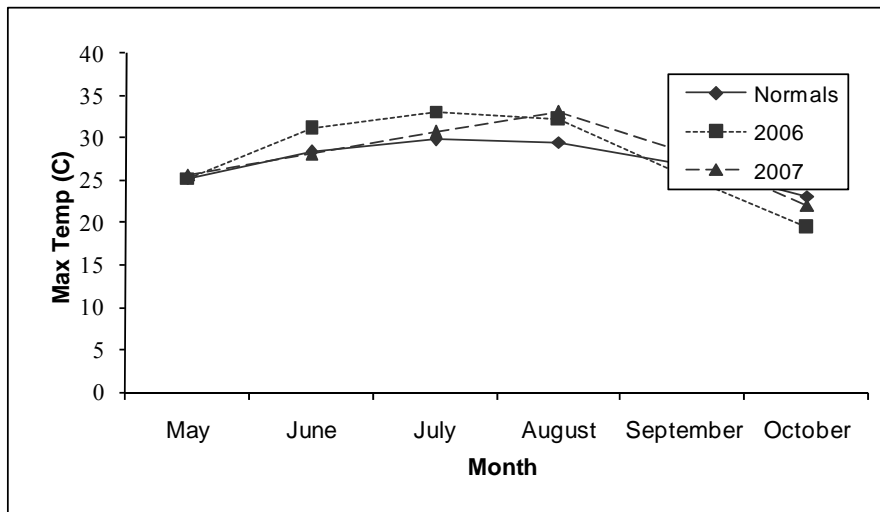


**Figure A.6.** Growing season minimum temperatures in °C for 30 year normals, 2006, and 2007 at the Ashland Scandia location.

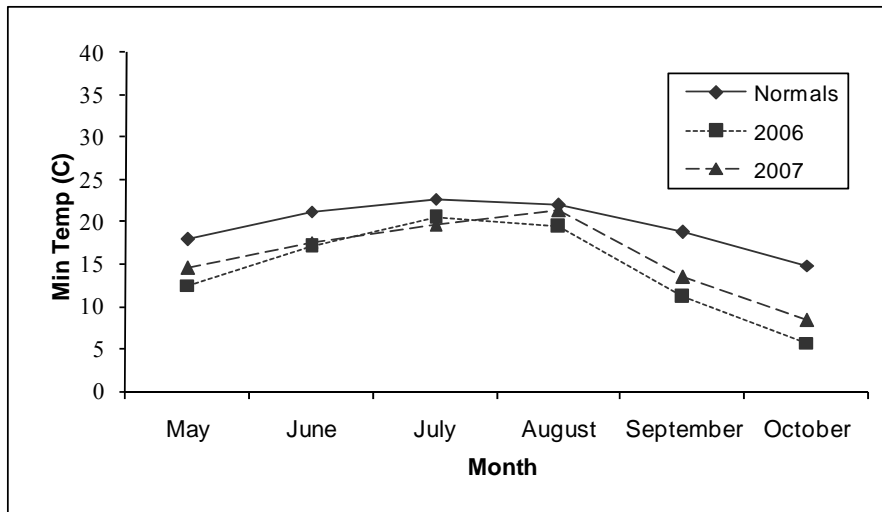
**Rossville Location**



**Figure A.7.** Growing season precipitation in mm for 30 year normals, 2006, and 2007 at the Rossville location.

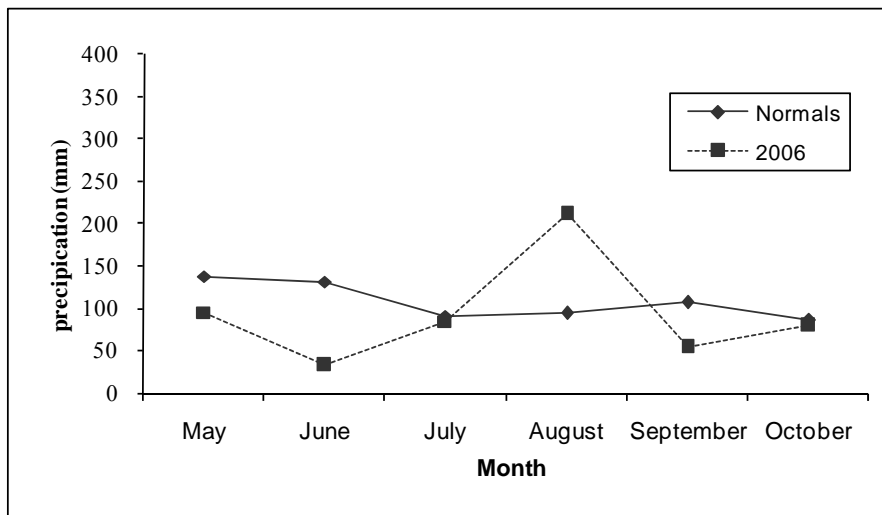


**Figure A.8.** Growing season maximum temperatures in °C for 30 year normals, 2006, and 2007 at the Rossville location.

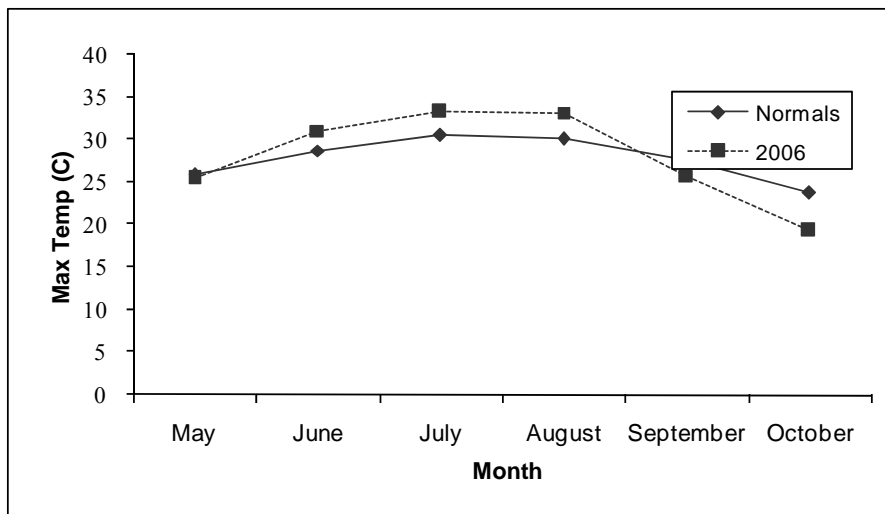


**Figure A.9.** Growing season minimum temperatures in °C for 30 year normals, 2006, and 2007 at the Rossville location.

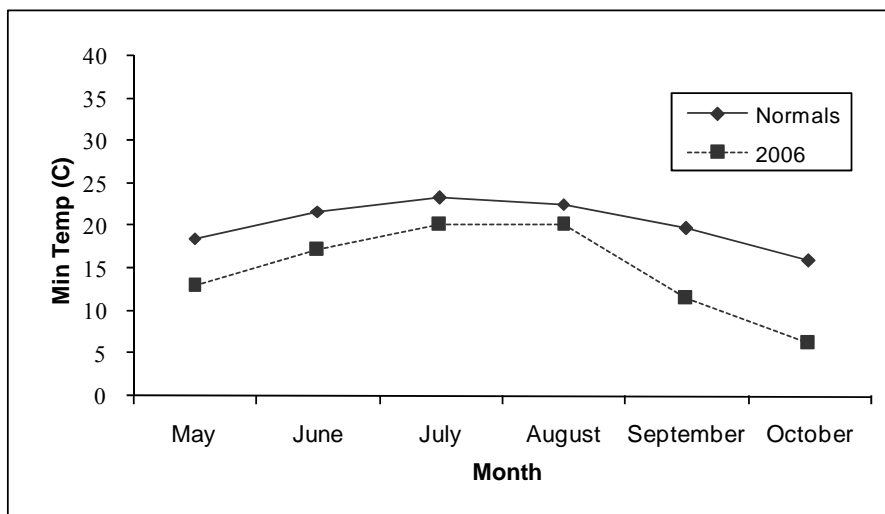
*Ottawa Location*



**Figure A.10.** Growing season precipitation in mm for 30 year normals, 2006, and 2007 at the Ottawa location.



**Figure A.11.** Growing season maximum temperatures in °C for 30 year normals, 2006, and 2007 at the Ottawa location.



**Figure A.12.** Growing season minimum temperatures in °C for 30 year normals, 2006, and 2007 at the Rossville location.

## Appendix B - Statistics Code (SAS)

### *Basic SAS Code used for Chapter 2 and Chapter 3*

```
input Year Location $ Plotrep variety $ rate Mn1 Mn2 Mn3 seed yield;
datalines;
proc print;
run;
proc print;
quit;
Proc sort;
by location year;
proc mixed;
by location year;
class rep variety rate;
model Mn1= variety|rate/ddfm = satterth;
random rep rep*variety;
lsmeans variety|rate/pdiff;
run;
```

### *Basic SAS Code used for Chapter 3*

```
data;
input Year Location $ Plot rep date variety part grams tot_N P K Mg Ca S Zn Mn Cu Fe B;
datalines;
proc print;
run;
proc sort;
by year location part;
proc mixed;
by year location part;
class rep date variety;
model tot_N = variety|date/ddfm = satterth;
random rep rep*variety;
lsmeans variety|date/pdiff;
run;
```

### *Basic SAS Code used for Chapter 4*

```
title 'Greenhousetotalaccumulation';
data greenhouse;
input rep plot glyphosate rate mn;
cards;
run;
proc print;
quit;
proc glm data=greenhouse;
class rep glyphosate rate;
model mn = rep glyphosate | rate;
random rep/test;
means glyphosate rate/lsd lines;
lsmeans glyphosate * rate / pdiff;
```

```
lsmeans glyphosate * rate / stderr;  
quit;
```

## Appendix C – R6 Mn Concentrations for Mn Rate Study

**Table C.1.** Manganese concentrations in soybean leaf tissue at growth stage R6 for conventional (CV) and glyphosate-resistant (GR) sister lines. (LSD for Mn rate by soybean near isoline interactions).

Year	Mn Rate	Ashland		Rossville		Scandia		Ottawa		Manhattan	
		Bottoms		CV	GR	CV	GR	CV	GR	CV	GR
----- kg/ha -----											
2006	0	78.2	72.2	82.5	80.0	68.2	68.0	82.0	86.5	NA <sup>†</sup>	NA <sup>†</sup>
	0.22	74.2	71.7	80.0	79.0	NA <sup>†</sup>	NA <sup>†</sup>	85.7	86.0	NA <sup>†</sup>	NA <sup>†</sup>
	0.44	72.2	83.0	83.5	85.0	NA <sup>†</sup>	NA <sup>†</sup>	83.7	81.7	NA <sup>†</sup>	NA <sup>†</sup>
	2.8	73.2	74.5	85.2	85.2	71.4	76.0	83.0	86.5	NA <sup>†</sup>	NA <sup>†</sup>
	5.6	73.7	79.5	83.2	88.0	76.3	63.5	81.2	85.2	NA <sup>†</sup>	NA <sup>†</sup>
	8.4	72.7	78.0	85.0	78.5	77.1	66.0	83.5	88.2	NA <sup>†</sup>	NA <sup>†</sup>
LSD (0.05)		NS		NS		NS		NS			
2007	0	113.7	113.2	88.7	89.0	141.7	124.0	NA <sup>†</sup>	NA <sup>†</sup>	84.0	60.5
	0.22	116.7	121.2	99.0	91.2	130.7	115.0	NA <sup>†</sup>	NA <sup>†</sup>	63.0	34.8
	0.44	116.5	118.2	87.7	91.5	102.7	77.0	NA <sup>†</sup>	NA <sup>†</sup>	66.5	61.8
	2.8	120.2	121.7	84.2	91.7	112.0	112.9	NA <sup>†</sup>	NA <sup>†</sup>	50.7	69.2
	5.6	122.7	128.5	91.5	94.2	101.2	143.9	NA <sup>†</sup>	NA <sup>†</sup>	52.7	33.2
	8.4	131.0	116.7	97.5	90.2	106.4	117.4	NA <sup>†</sup>	NA <sup>†</sup>	68.5	57.3
LSD (0.05)		NS		NS		NS				NS	

## Appendix D – Plant Height and Biomass Data

**Table D.1.** Soybean plant height (cm) in conventional (CV) and glyphosate-resistant (GR) soybean at growth stage R1 at all locations during 2006 and 2007. (LSD for Mn rate by soybean near isoline interactions).

Year	Mn Rate	Ashland		Rossville		Scandia		Ottawa		Manhattan	
		Bottoms		CV	GR	CV	GR	CV	GR	CV	GR
----- cm -----											
2006	0	34.2	32.6	38.6	39.9	65.2	68.4	41.2	40.5	NA <sup>†</sup>	NA <sup>†</sup>
	0.22	35.6	35.3	40.3	39.6	NA <sup>†</sup>	NA <sup>†</sup>	39.8	39.3	NA <sup>†</sup>	NA <sup>†</sup>
	0.44	34.7	34.3	41.0	40.5	NA <sup>†</sup>	NA <sup>†</sup>	40.3	37.4	NA <sup>†</sup>	NA <sup>†</sup>
	2.8	32.5	35.0	38.7	34.8	69.2	65.5	39.4	39.1	NA <sup>†</sup>	NA <sup>†</sup>
	5.6	35.9	35.1	37.7	41.4	68.7	67.6	38.5	37.7	NA <sup>†</sup>	NA <sup>†</sup>
	8.4	33.9	31.8	40.5	39.5	66.1	66.0	40.0	41.5	NA <sup>†</sup>	NA <sup>†</sup>
LSD (0.05)		NS		NS		NS		NS			
2007	0	56.4	58.4	55.2	56.6	71.2	67.3	NA <sup>†</sup>	NA <sup>†</sup>	48.7	47.3
	0.22	54.9	58.4	56.6	59.9	72.3	67.3	NA <sup>†</sup>	NA <sup>†</sup>	48.8	48.7
	0.44	57.3	62.0	56.5	59.3	70.3	67.5	NA <sup>†</sup>	NA <sup>†</sup>	48.3	48.7
	2.8	54.7	62.4	54.4	58.9	70.7	67.4	NA <sup>†</sup>	NA <sup>†</sup>	52.7	45.1
	5.6	58.5	63.7	57.7	60.8	72.5	66.4	NA <sup>†</sup>	NA <sup>†</sup>	47.5	49.0
	8.4	57.0	59.5	57.8	59.0	70.8	67.3	NA <sup>†</sup>	NA <sup>†</sup>	49.2	48.2
LSD (0.05)		NS		NS		NS				NS	

† Data not available

**Table D.2.** Analysis of variance (ANOVA) p-values from F-tests for near isoline (GR and CV) and Mn rate (foliar and granular Mn fertilizer) effects on plant height at growth stage R1.

		Ashland	Rossville	Scandia	Ottawa	Manhattan
		Bottoms				
2006	near isoline	0.892	0.879	0.634	0.614	NA <sup>†</sup>
	rate	0.643	0.585	0.276	0.187	NA <sup>†</sup>
	near isoline*rate	0.843	0.662	0.060	0.632	NA <sup>†</sup>
2007	near isoline	0.002	0.002	<0.001	NA <sup>†</sup>	0.675
	rate	0.529	0.189	0.826	NA <sup>†</sup>	0.999
	near isoline*rate	0.800	0.850	0.235	NA <sup>†</sup>	0.503

† Data not available



**Table D.3.** Soybean plant height (cm) in conventional (CV) and glyphosate-resistant (GR) soybean at growth stage R3 at all locations during 2006 and 2007. (LSD for Mn rate by soybean near isoline interactions).

Year	Mn Rate	Ashland		Rossville		Scandia		Ottawa		Manhattan	
		Bottoms	GR	CV	GR	CV	GR	CV	GR	CV	GR
----- cm -----											
2006	0	84.5	82.8	73.8	75.5	113.3	100.3	84.1	86.8	NA <sup>†</sup>	NA <sup>†</sup>
	0.22	80.2	80.8	71.7	74.3	NA <sup>†</sup>	NA <sup>†</sup>	84.9	86.1	NA <sup>†</sup>	NA <sup>†</sup>
	0.44	82.9	80.5	74.5	74.7	NA <sup>†</sup>	NA <sup>†</sup>	83.2	85.3	NA <sup>†</sup>	NA <sup>†</sup>
	2.8	81.4	82.0	73.6	72.5	101.0	103.3	86.4	82.7	NA <sup>†</sup>	NA <sup>†</sup>
	5.6	79.8	82.9	76.6	77.9	101.5	105.1	83.4	83.2	NA <sup>†</sup>	NA <sup>†</sup>
	8.4	84.8	80.4	73.2	74.3	100.5	104.2	85.5	87.0	NA <sup>†</sup>	NA <sup>†</sup>
LSD (0.05)		NS		NS		NS		NS			
2007	0	82.8	88.7	98.4	100.7	120.3	115.1	NA <sup>†</sup>	NA <sup>†</sup>	86.7	82.6
	0.22	83.3	89.4	100.5	97.0	123.2	117.2	NA <sup>†</sup>	NA <sup>†</sup>	84.8	78.1
	0.44	83.1	85.5	97.9	99.9	121.0	117.4	NA <sup>†</sup>	NA <sup>†</sup>	85.4	82.7
	2.8	84.5	85.2	97.0	96.5	119.4	120.5	NA <sup>†</sup>	NA <sup>†</sup>	87.1	80.4
	5.6	85.9	90.6	101.3	97.4	120.5	122.0	NA <sup>†</sup>	NA <sup>†</sup>	85.5	83.6
	8.4	84.7	87.2	98.3	99.2	118.2	113.7	NA <sup>†</sup>	NA <sup>†</sup>	85.2	81.3
LSD (0.05)		NS		NS		NS				NS	

† Data not available

**Table D.4.** Analysis of variance (ANOVA) p-values from F-tests for near isoline (GR and CV) and Mn rate (foliar and granular Mn fertilizer) effects on plant height at growth stage R3.

		Ashland	Rossville	Scandia	Ottawa	Manhattan
		Bottoms				
2006	near isoline	0.539	0.620	0.741	0.774	NA <sup>†</sup>
	rate	0.724	0.443	0.479	0.622	NA <sup>†</sup>
	near isoline*rate	0.536	0.974	0.203	0.509	NA <sup>†</sup>
2007	near isoline	0.238	0.653	0.082	NA <sup>†</sup>	0.520
	rate	0.726	0.509	0.425	NA <sup>†</sup>	0.901
	near isoline*rate	0.900	0.180	0.608	NA <sup>†</sup>	0.946

† Data not available

**Table D.5.** Soybean plant height (cm) in conventional (CV) and glyphosate-resistant (GR) soybean at growth stage R6 at all locations during 2006 and 2007. (LSD for Mn rate by soybean near isoline interactions).

Year	Mn Rate	Ashland		Rossville		Scandia		Ottawa		Manhattan	
		Bottoms		CV	GR	CV	GR	CV	GR	CV	GR
----- cm -----											
2006	0	98.5	104.3	89.3	93.7	114.5	114.5	85.2	87.5	NA <sup>†</sup>	NA <sup>†</sup>
	0.22	97.2	101.9	91.1	91.9	NA <sup>†</sup>	NA <sup>†</sup>	85.0	84.9	NA <sup>†</sup>	NA <sup>†</sup>
	0.44	93.9	97.5	94.3	95.4	NA <sup>†</sup>	NA <sup>†</sup>	83.6	84.8	NA <sup>†</sup>	NA <sup>†</sup>
	2.8	98.5	102.4	88.1	89.7	111.8	115.1	86.2	82.5	NA <sup>†</sup>	NA <sup>†</sup>
	5.6	93.7	100.1	91.8	94.1	114.7	113.4	83.7	82.2	NA <sup>†</sup>	NA <sup>†</sup>
	8.4	93.9	105.4	92.1	93.1	119.0	112.3	85.6	86.8	NA <sup>†</sup>	NA <sup>†</sup>
LSD (0.05)		NS		NS		NS		NS			
2007	0	113.3	115.2	112.4	110.0	115.0	110.4	NA <sup>†</sup>	NA <sup>†</sup>	90.3	100.7
	0.22	111.7	114.9	117.7	114.4	114.1	113.8	NA <sup>†</sup>	NA <sup>†</sup>	98.2	90.7
	0.44	117.2	112.3	112.5	114.3	115.7	110.9	NA <sup>†</sup>	NA <sup>†</sup>	93.7	92.7
	2.8	115.8	113.3	114.7	112.4	116.9	112.5	NA <sup>†</sup>	NA <sup>†</sup>	103.9	91.6
	5.6	119.4	114.5	112.0	112.8	117.6	112.4	NA <sup>†</sup>	NA <sup>†</sup>	105.1	91.7
	8.4	117.9	115.7	116.5	111.9	115.2	113.0	NA <sup>†</sup>	NA <sup>†</sup>	100.0	93.6
LSD (0.05)		NS		NS		NS				NS	

† Data not available

**Table D.6.** Analysis of variance (ANOVA) p-values from F-tests for near isoline (GR and CV) and Mn rate (foliar and granular Mn fertilizer) effects on plant height at growth stage R6.

		Ashland	Rossville	Scandia	Ottawa	Manhattan
		Bottoms				
2006	near isoline	<0.001	0.230	0.832	0.958	NA <sup>†</sup>
	rate	0.153	0.363	0.621	0.424	NA <sup>†</sup>
	near isoline*rate	0.576	0.984	0.073	0.607	NA <sup>†</sup>
2007	near isoline	0.309	0.205	<0.001	NA <sup>†</sup>	0.661
	rate	0.682	0.394	0.445	NA <sup>†</sup>	0.923
	near isoline*rate	0.514	0.698	0.305	NA <sup>†</sup>	0.282

† Data not available

**Table D.7.** Soybean plant biomass in g/plot for conventional (CV) and glyphosate-resistant (GR) soybean at growth stage R1 at most locations during 2006 and 2007. (*p*-value is for near isoline comparison).

		Ashland Bottoms	Rossville	Scandia	Ottawa	Manhattan
2006		g of biomass/sampled plot				
	CV	84.7	89.5	NA <sup>†</sup>	87.3	NA <sup>†</sup>
	GR	78.3	65.4	NA <sup>†</sup>	90.8	NA <sup>†</sup>
	near isoline <i>p</i> -value	0.584	0.176	NA <sup>†</sup>	0.527	NA <sup>†</sup>
2007		g of biomass/sampled plot				
	CV	113.0	88.5	137.5	NA <sup>†</sup>	186.0
	GR	106.5	176.2	288.5	NA <sup>†</sup>	183.2
	near isoline <i>p</i> -value	0.436	0.006	0.041	NA <sup>†</sup>	0.859

† Data not available

**Table D.8.** Soybean plant biomass in g/plot for conventional (CV) and glyphosate-resistant (GR) soybean at growth stage R3 at most locations during 2006 and 2007. (*p*-value is for near isoline comparison).

		Ashland Bottoms	Rossville	Scandia	Ottawa	Manhattan
2006		g of biomass/sampled plot				
	CV	305.3	226.0	NA <sup>†</sup>	364.1	NA <sup>†</sup>
	GR	296.3	199.8	NA <sup>†</sup>	279.5	NA <sup>†</sup>
	near isoline <i>p</i> -value	0.851	0.665	NA <sup>†</sup>	0.023	NA <sup>†</sup>
2007		g of biomass/sampled plot				
	CV	216.2	155.2	306.0	NA <sup>†</sup>	278.5
	GR	245.5	323.2	505.2	NA <sup>†</sup>	286.2
	near isoline <i>p</i> -value	0.172	0.060	0.001	NA <sup>†</sup>	0.830

† Data not available

**Table D.9.** Soybean plant biomass in g/plot for conventional (CV) and glyphosate-resistant (GR) soybean at growth stage R6 at most locations during 2006 and 2007. (*p*-value is for near isoline comparison).

		Ashland Bottoms	Rossville	Scandia	Ottawa	Manhattan
2006		g of biomass/sampled plot				
	CV	163.4	136.1	NA <sup>†</sup>	195.9	NA <sup>†</sup>
	GR	191.4	204.1	NA <sup>†</sup>	189.0	NA <sup>†</sup>
	near isoline <i>p</i> - value	0.165	0.035	NA <sup>†</sup>	0.206	NA <sup>†</sup>
2007						
	CV	106.0	60.3	107.8	NA <sup>†</sup>	252.1
	GR	101.9	147.2	199.42	NA <sup>†</sup>	213.9
	near isoline <i>p</i> - value	0.368	<0.0001	0.001	NA <sup>†</sup>	0.015

† Data not available